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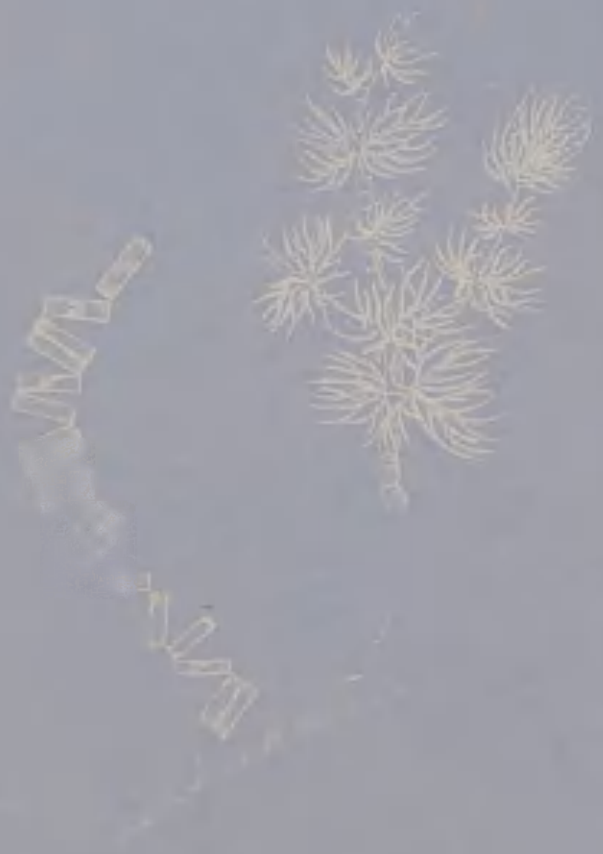

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PREFACE

This issue is dedicated to Pierre Bourrelly who was editor of the journal *Cryptogamie, Algologie*, and who was regarded as one of the foremost experts on freshwater algae of his time. His books remain indispensable tools for freshwater phycologists. The article by A. Couté (In Memoriam. *Cryptogamie, Algologie*, 1996, 17 (1): 1-17) includes a complete list of his publications. His correspondence, involving over 3000 letters, is preserved at PC and reflects the importance of his contribution to Phycology. He was an exceptionally kind man, always available for anyone, researcher or student, to whom he opened his large library, including an extensive, unvaluable file of taxonomic data on each species of freshwater algae, and shared his encyclopedic knowledge. He also knew the algal collections housed at PC very well and was very helpful in guiding colleagues through these collections. He was not only a phycologist but also a "naturalist" and was an excellent identificator of vascular plants. When I was hired by the Muséum National d'Histoire Naturelle (1991), he was actively pursuing his "second career" as a retired professor, updating his books! His humour was intact, and though I was one of those frightful people who dealt with "uninteresting" marine algae, we had daily morning talks which I will remember fondly. It has been a delight to edit this dedicated issue and join colleagues around the world who have contributed to it and rendered hommage to him.

The Editor:

Bruno de Reviers

1. Prof. Dr Ludwig Kies dedicated an article to Pierre Bourrelly in *Limnologica*: Kies L., 1997 – Distribution, biomass and production of plancktonic and benthic algae in the Elbe estuary. *Limnologica* 27 (1): 55-64.

COMPARATIVE ULTRASTRUCTURE OF CHLOROPLASTS IN THE SUBGENUS *EUGLENA* (EUGLENOPHYTA): TAXONOMIC SIGNIFICANCE*

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ABSTRACT — The taxonomic disposition of *Euglena geniculata* Dujardin and *E. myxocylindracea* Bold & MacEntee has been uncertain. Our electron-microscopic investigations now show that these two organisms have a chloroplast structure and morphology similar to those of other species presently classified in the Subgenus *Euglena*, *i.e.*, *E. stellata* Mainx, *E. tristella* Chu and *E. viridis* Ehrenberg. In this subgenus, chloroplasts are axial, elongate or stellate, with pyrenoids. The lobes of the stellate plastids branch repeatedly, giving rise to very narrow ribbons that extend to subpellicular regions and flank the nucleus, thus occupying much of the cytoplasm. A "paramylon center" consisting of many paramylon grains surrounds the pyrenoid and renders it invisible in the light microscope. Centrally located in the plastid, the pyrenoid matrix is penetrated by a few thylakoid pairs. Maintenance of *Euglena* strains on synthetic media in culture for many years does not cause visible changes in chloroplast morphology or ultrastructure.

RÉSUMÉ — La position taxinomique de *Euglena geniculata* Dujardin et celle de *E. myxocylindracea* Bold & MacEntee est incertaine. Nos études en microscopie électronique montrent que ces deux organismes possèdent une morphologie et une structure chloroplastiques semblables à celles d'autres espèces actuellement placées dans le sous-genre *Euglena*, *i.e.*, *E. stellata* Mainx, *E. tristella* Chu and *E. viridis* Ehrenberg. Dans ce sous-genre, les chloroplastes sont axiaux, allongés ou étoilés, et possèdent des pyrénoïdes. Les lobes des plastes étoilés sont plusieurs fois ramifiés, donnant naissance à des rubans très étroits qui s'étendent à la région sous-pelliculaire et bordent le noyau, occupant ainsi la plus grande partie du cytoplasme. Un « *paramylon center* », constitué de nombreux grains de paramylon, entoure le pyrénoïde et le rend invisible au microscope optique. Située au centre du plaste, la matrice du pyrénoïde est pénétrée par quelques paires de thylacoïdes. Le maintien de souches d'*Euglena* en culture, dans des milieux synthétiques, pendant de nombreuses années, ne provoque pas de modifications visibles dans la morphologie ou l'ultrastructure des chloroplastes. (Traduit par la Rédaction)

KEY WORDS: Chloroplast, *Euglena*, *E. geniculata*, *E. myxocylindraceu*, *E. stellata*, *E. viridis*, freshwater algae, pyrenoid, systematics, taxonomy, ultrastructure.

* We dedicate this paper to the memory of Professor P. Bourrelly. The strain of *Euglena viridis* Ehrenb. used in this investigation was isolated by him.

INTRODUCTION

The identification of intra-generic taxa of *Euglena* has been based largely on well-known morphological characters at the light-microscopic level, but especially on the historically important criterion of chloroplasts (their number, size, location; presence or absence of pyrenoids; number, morphology and location of paramylon grains). Many authorities have acknowledged chloroplast characteristics as the major criteria for the establishment of an intra-generic classification for *Euglena* (Dangeard, 1901; Lemmermann, 1913; Chu, 1946; Gojdics, 1953; Huber-Pestalozzi, 1955; Pringsheim, 1956). For most of the species of *Euglena*, however, details about the chloroplast are insufficiently known and described, a particular concern for taxa of the subgenus *Euglena* Ehrenb. (Zakryś, 1986), which is characterized by axial chloroplasts. Often obscured by other cellular structures and organelles, the plastids are not readily discernible or only faintly so in the light microscope, thus necessitating more detailed examination by transmission electron microscopy.

Of special interest for this investigation are two species tentatively classified in the Subgenus *Euglena* for which chloroplast ultrastructure is not described: *E. myxocylindracea* and *E. geniculata.* Light-microscopic observations to date on these organisms have not allowed definitive determinations about the number and morphology of the plastids, including the presence of pyrenoids. For comparative purposes two other species from the Subgenus *Euglena* (*E. stellata* and *E. viridis*) have been studied concurrently in order to document our findings. Based on earlier TEM studies it is known that these two species have single, stellate, axial plastids with pyrenoids (Dragos *et al.*, 1979). Those investigations, however, were carried out on natural populations, whereas our study is based on *Euglena* strains obtained from algal culture collections where the organisms have been maintained on synthetic media for many years. It is well known that even moderate adverse environmental conditions, such as excessive density of the population or exhaustion of nutrients, can occur in natural populations, as well as in culture and may affect chloroplast structure and morphology on a permanent basis.

The purpose of this investigation is twofold:

1. To document chloroplast number and morphology in two species of the Subgenus *Euglena* for which ultrastructural details are presently unreported (*E. myxocylindracea* and *E. geniculata*) and to compare these data to other known species of the Subgenus (*E. stellata* and *E. viridis*), also obtained from culture collections and processed for TEM with identical procedures.

2. To determine whether long-term culturing on synthetic media may result in permanent changes in chloroplast structure.

MATERIALS AND METHODS

Cultures.

All strains of *Euglena* were obtained from either the Sammlung von Algenkulturen (SAG), Göttingen, Germany, or The Culture Collection of Algae, University of Texas (UTEX), Austin, Texas, U.S.A.: *E. geniculata* Dujardin, strain #1224-4g (SAG —

isolated by E.G. Pringsheim); *E. stellata* Mainx, strain #1224-14 (SAG — isolated by F. Mainx); *E. viridis* Ehrenb., strain #1224-17b (SAG — isolated by P. Bourrelly); and *E. myxocylindracea* Bold & MacEntee, strain #1989 (UTEX — isolated by the authorities). Clones derived from each strain were cultured in liquid media (Starr, 1978) and under identical conditions in a growth chamber maintained at 19° C and 16:8 hr L/D, ca 27 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent tubes. Two-week-old cultures were used for the electron-microscopic studies.

Transmission electron microscopy.

Actively swimming cells were harvested by low-speed centrifugation (1500 g) and fixed in 2% glutaraldehyde buffered with 0,05 M sodium cacodylate, pH 7.2, for 1 hr at 4° C. After several rinses in buffer, the samples were post-fixed in buffered 2% OsO_4 also for 1 hr at 4° C, followed by dehydration in a graded ethanol/acetone series and embedment in Spurr's low-viscosity resin. Thin sections were cut with a diamond knife on a Reichert OMU-3 ultramicrotome and were stained sequentially with uranyl acetate and lead citrate, prior to examination in a Hitachi H-600 transmission electron microscope, operating at 75-100 kV.

RESULTS AND DISCUSSION

Euglena myxocylindracea. The chloroplast is a single, stellate, axial structure with a centrally located large pyrenoid (Figs 1-4). An integral part of the plastid, the pyrenoid is not surrounded by a defining membrane and is readily identifiable by its matrix, which is penetrated by a few thylakoid pairs that are continuous with the typical euglenoid triplet lamellae (Figs 5-7). The central portion of the chloroplast, containing the pyrenoid, is situated anterior to the nucleus. The pyrenoid region itself is often covered by other cellular organelles and numerous paramylon grains, termed a "paramylon center." The peripheral portions of the plastid may be divided repeatedly into narrow, ribbon-like branches that extend radially to the subpellicular regions at the cell surface, thus filling the cytoplasmic areas anterior and posterior to the nucleus (Diagram 1, Figs 1-4).

Euglena geniculata. Two axial, stellate chloroplasts of somewhat different sizes occupy the cell, the larger one anterior to the nucleus, the smaller one posterior to it. Each chloroplast contains a large central pyrenoid (Figs 19, 20). Other details of chloroplast and pyrenoid shape and structure are similar, even identical to those of *E. myxocylindracea* (Figs 21, 22, cf. Figs 1-4).

Euglena stellata and *Euglena viridis.* Similar observations of chloroplast and pyrenoid structure were made for *E. stellata* (Figs 8-13) and *E. viridis* (Figs 14-18). Long-term culture on synthetic media has not resulted in visible permanent changes in chloroplast structure. Our results on these two organisms are thus in agreement with those reported for specimens from natural populations (Dragos *et al.*, 1979).

Together with earlier investigations (Haller, 1959; Mignot, 1965, 1966; Buetow, 1968; Leedale, 1968; Dragos *et al.*, 1979; Péterfi *et al.*, 1979), our study shows convincingly that *E. myxocylindracea, E. geniculata, E. stellata, E. tristella,* and *E. viridis* have similar chloroplast structure, *i.e.*, stellate and axial, with centrally located pyrenoids (Diagram 1,

Fig 1) often obscured by a dense aggregation of paramylon grains termed a "paramylon center." The major difference among these species is chloroplast number: one per cell in *E. myxocylindracea, E. stellata,* and *E. viridis*; two in *E. geniculata*; three in *E. tristella.* Some differences in pyrenoid size between *E. stellata* and *E. viridis* were reported by Dragos *et. al.* (1979). In our opinion, however, such size differences are more likely to be due to interpopulational or even interclonal diversity, as shown for another species, *E. agilis* Carter (= *E. pisciformis* Klebs) (Zakryś & Kucharski, 1996; Zakryś *et al.*, 1996;), than to real species differences.

The hypothesis that *E. stellata* and *E. viridis* are not different species but rather clones of the same species is strongly supported by precise measurements of cell size, as well as by the great degree of DNA similarity among clones of both species (Zakryś *et al.*, in press). Nevertheless, additional research is essential for the requisite taxonomic revision of the group of species termed "*Euglena viridis* Group", Subgenus *Euglena,* in which *E. stellata* and *E. viridis*, and now *E. myxocylindracea* and *E. geniculata,* are classified (all species with morphological and chloroplast attributes similar to *E. viridis*). Such investigations will be the focus of future research.

SUMMARY

Euglena myxocylindracea has a single, stellate, axial chloroplast with a central pyrenoid, situated anterior to the nucleus, but often is not clearly discernible in the light microscope because of dense aggregations of paramylon grains termed a "paramylon center." *Euglena geniculata* has two stellate, axial chloroplasts, one anterior and one posterior to the nucleus. The centrally located pyrenoid in each plastid is frequently obscured by a paramylon center.

In both species, the pyrenoid matrix is penetrated by a few pairs of thylakoids, continuous with lamellar triplets in the chloroplast stroma. No major differences in chloroplast structure have been observed in all the species examined belonging to the Subgenus *Euglena.* Long-term culturing on synthetic media apparently has no visible, permanent effect on chloroplast structure or morphology.

ACKNOWLEDGEMENTS — This work was supported in part by a L. R. Hesler Visiting Professorship from the Department of Botany, University of Tennessee, Knoxville, and by grant BST from the Polish State Committee for Scientific Research. We thank Prof. Dr U. G. Schlösser, Sammlung von Algenkulturen (SAG), Göttingen, Germany, for providing several *Euglena* strains, and Dr John R. Dunlap and Mr Dick Williams, Life Sciences Microscopy Facility, UTK, for their interest and gracious technical assistance with the project.

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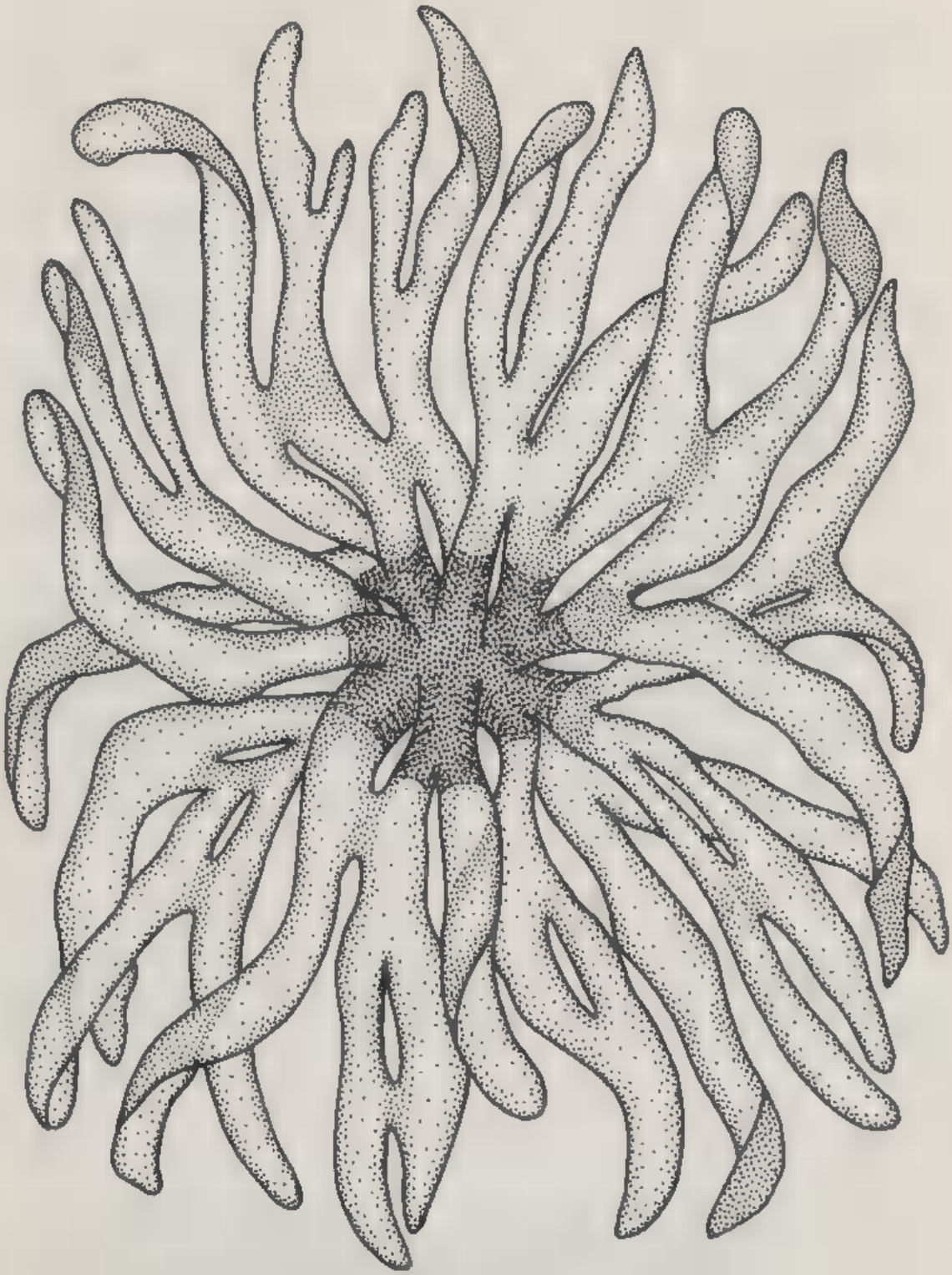


Diagram 1. Model of stellate chloroplast with centrally located pyrenoid (P).

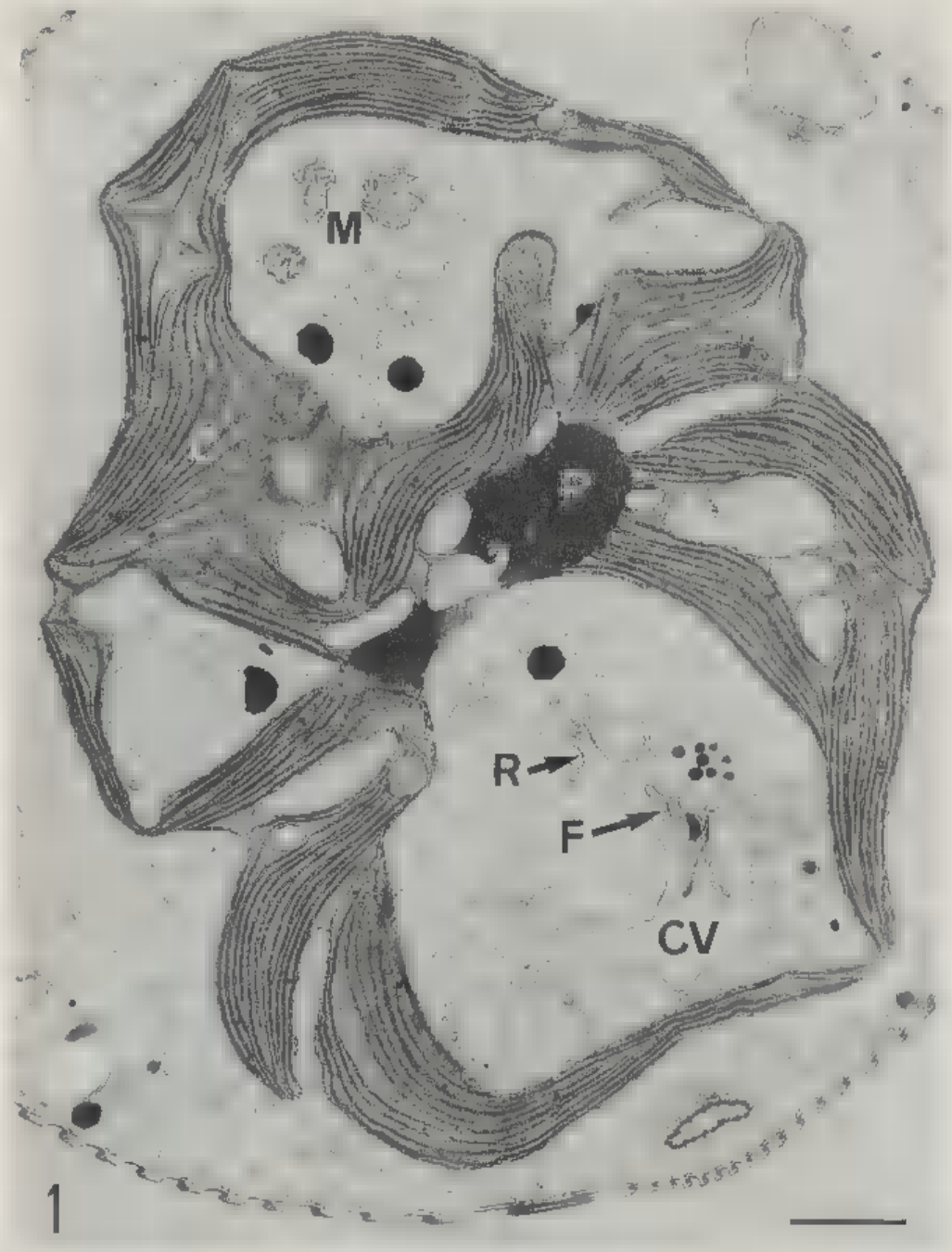
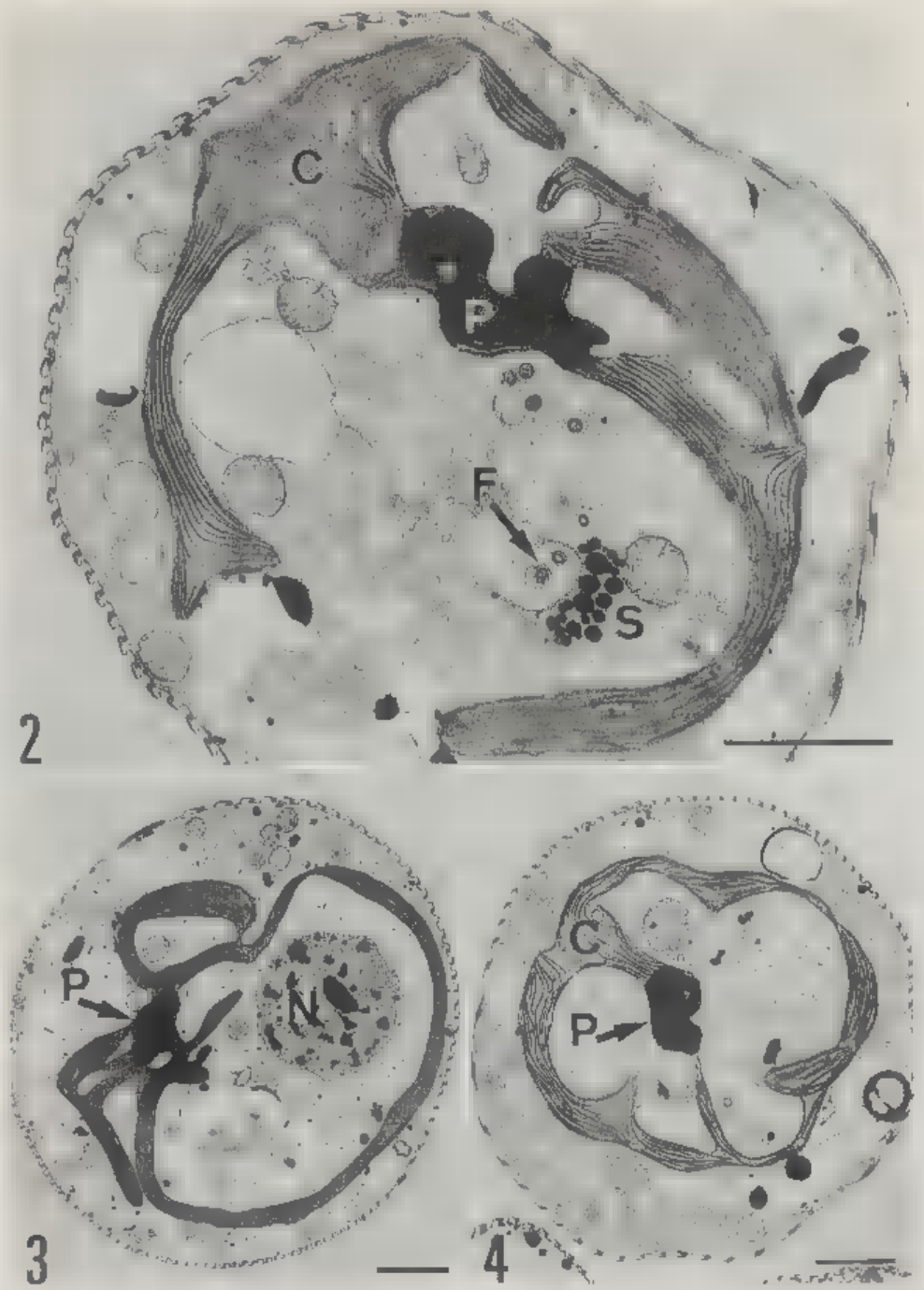
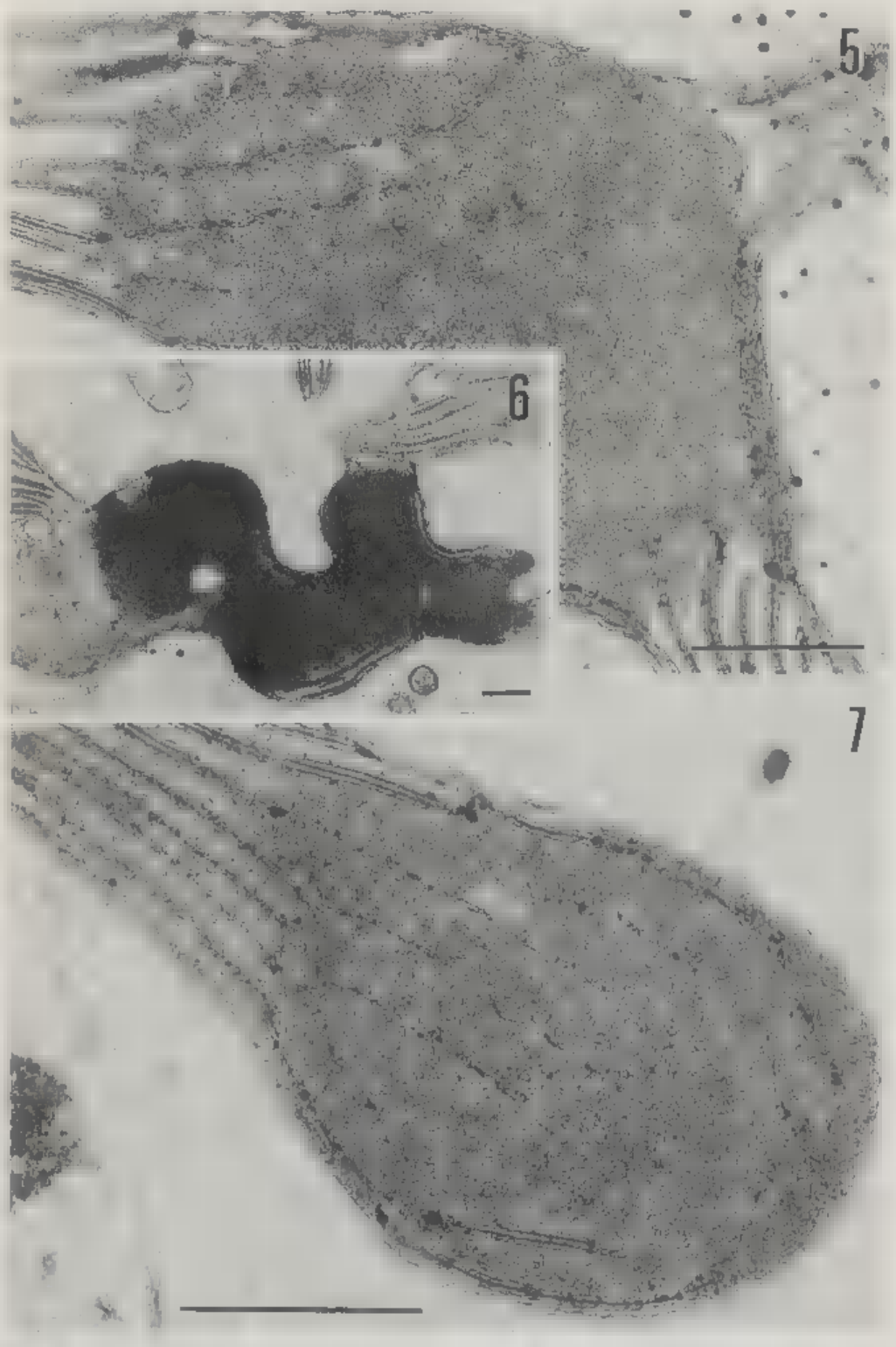


Fig. 1. *Euglena myxocylindracea.* Longitudinal oblique section shows typical cellular organization, including prominent single chloroplast (C) with pyrenoid (P), reservoir (R) with flagella (F), contractile vacuole (CV) and numerous mitochondria (M). Bar = 3 μm.



Figs 2-4. *Euglena myxocylindracea.* Transverse sections through various levels of cell show single chloroplast (C) with pyrenoid (P), nucleus (N), reservoir with flagella (F) and cytoplasmic stigma (S). Bars = 3 μm.



Figs 5-7. *Euglena nyxocylindracea.* Higher magnification of pyrenoids shows positional relationship of thylakoids to pyrenoid matrix. Some thylakoids extend into the matrix (cf. Figs 11,17, 21). Bars = 0,5 μm.

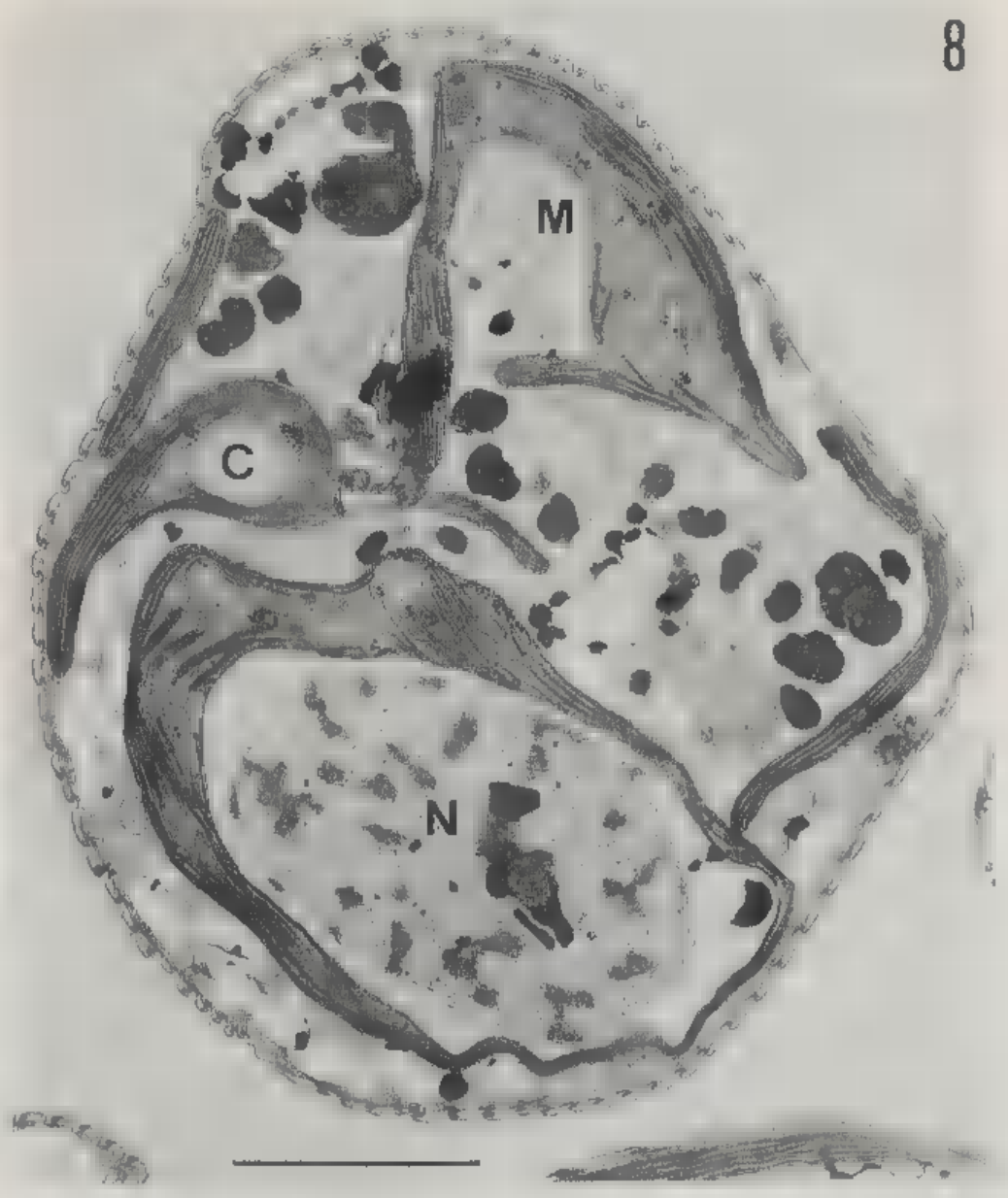
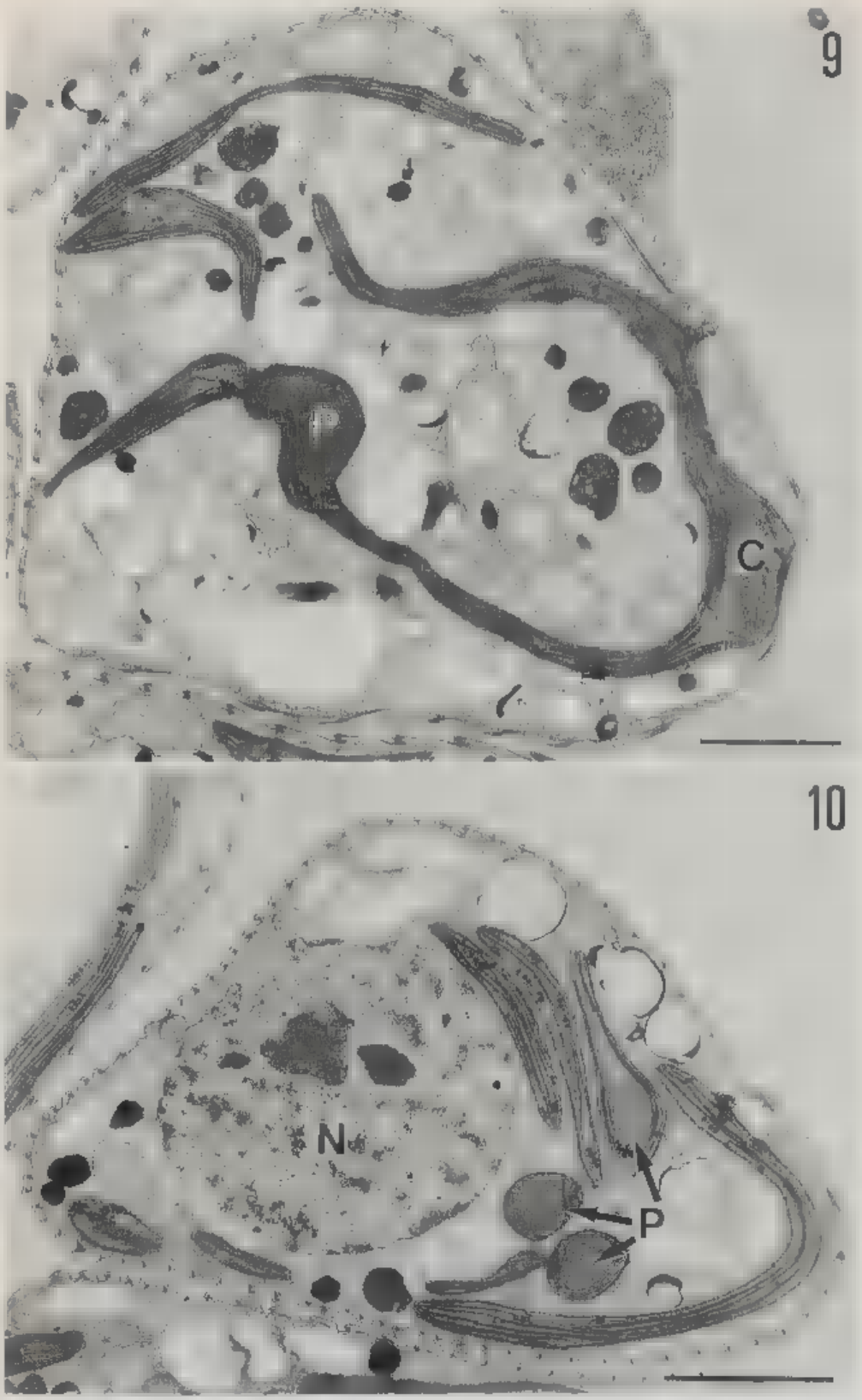
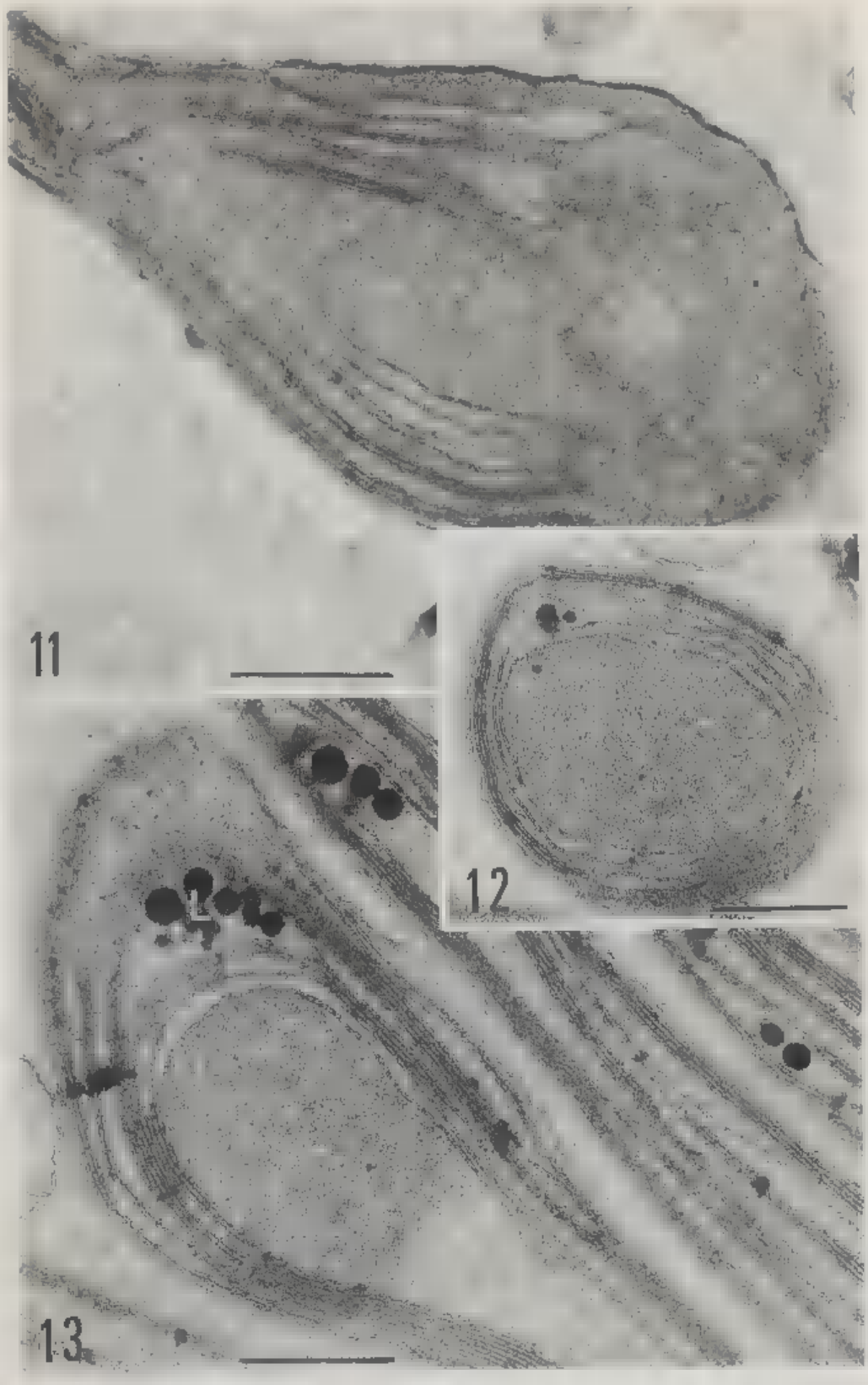


Fig. 8. *Euglena stellata*. Overview of cell in longitudinal oblique section showing chloroplast (C), nucleus (N), mitochondria (M), and numerous cytoplasmic vacuoles. Bar = 4 μm.

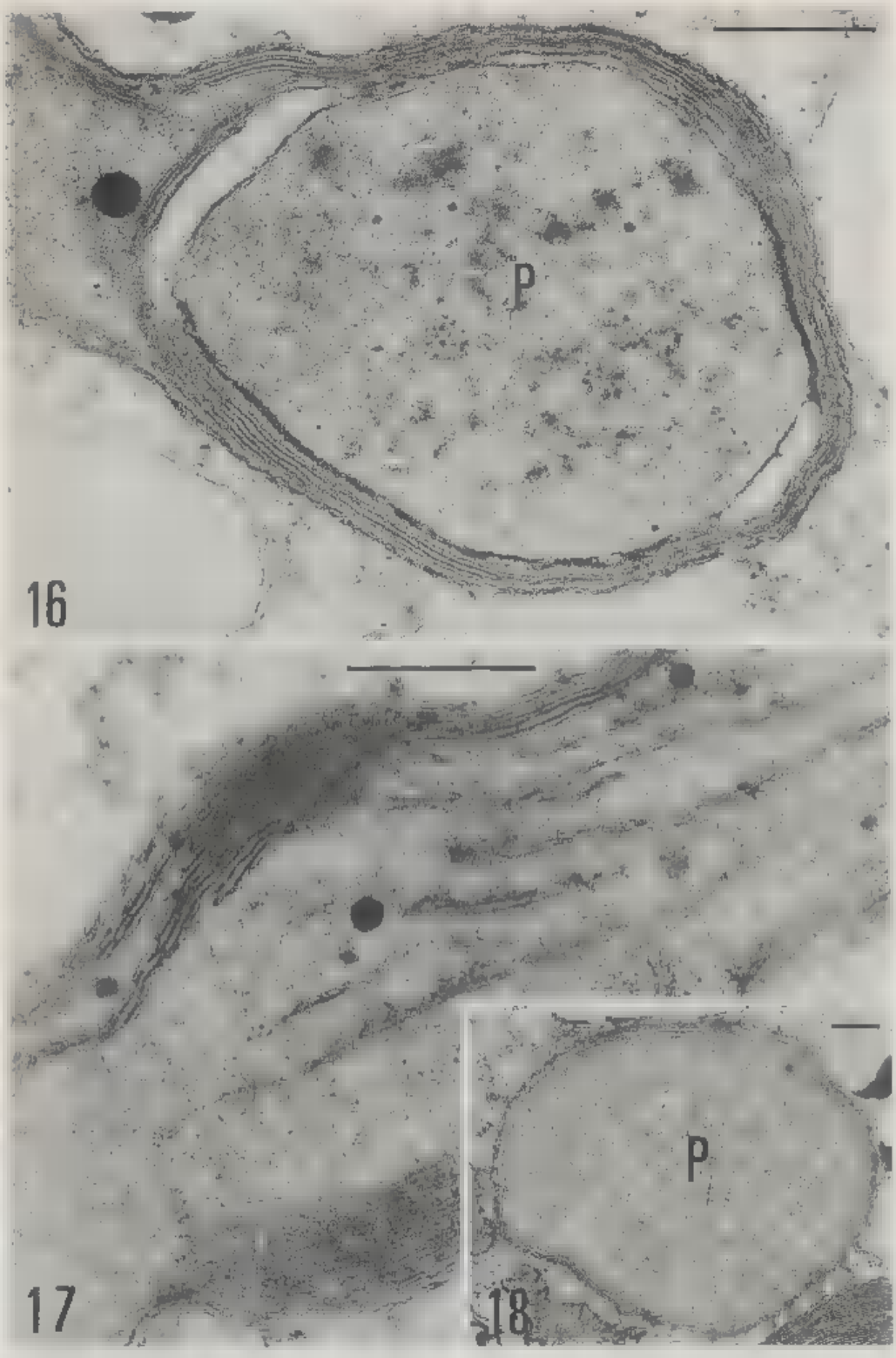


Figs 9-10. *Euglena stellata*. Transverse oblique sections at different levels showing multilobed chloroplasts (C), pyrenoids (P), nucleus (N), paramylon grains and cytoplasmic vacuoles. Bars = 3 μm.

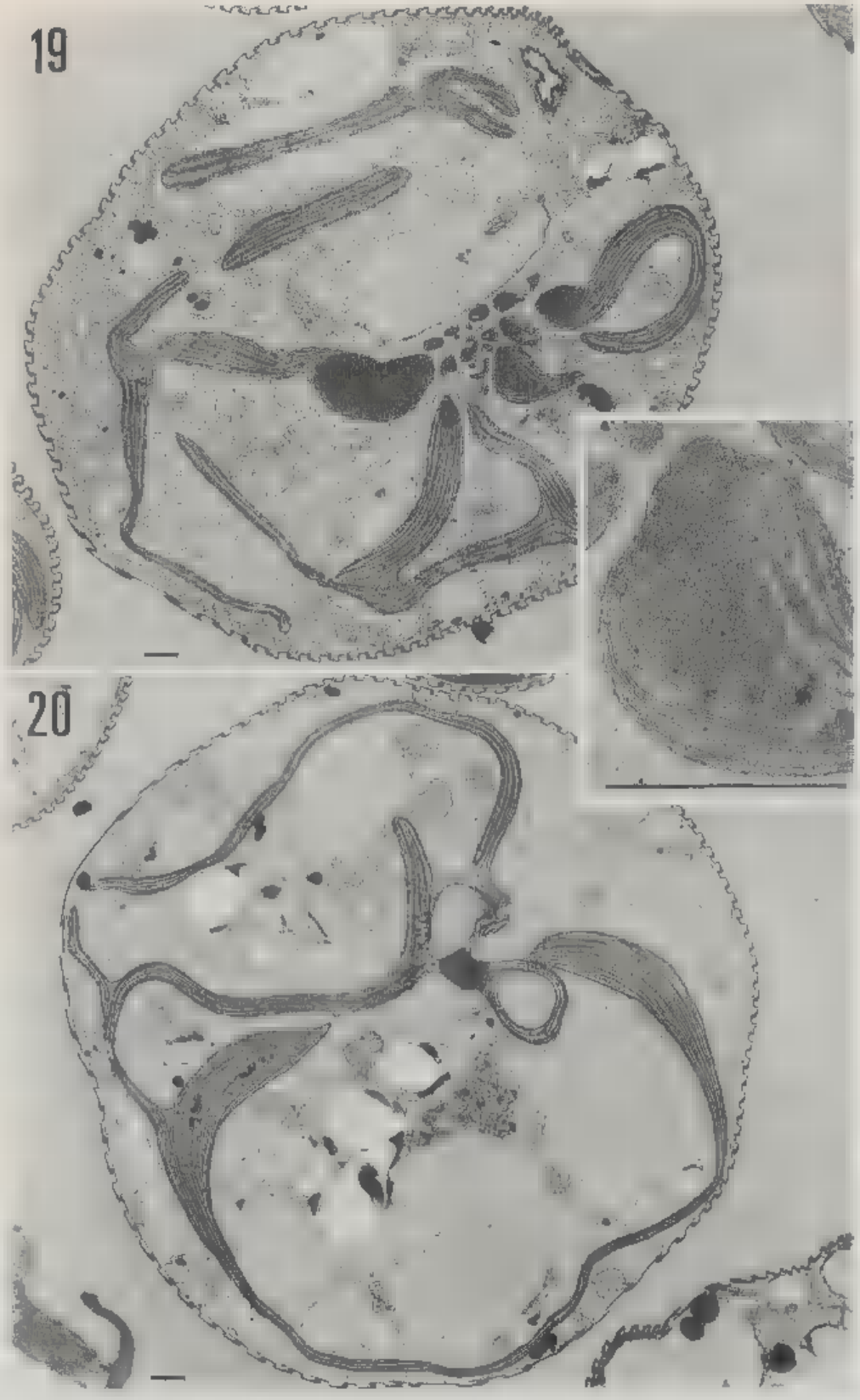


Figs 11-13. *Euglena stellata*. Higher magnification of pyrenoids shows matrix, peripheral thylakoids, and lipid granules (L). Bars = 0,5 µm.

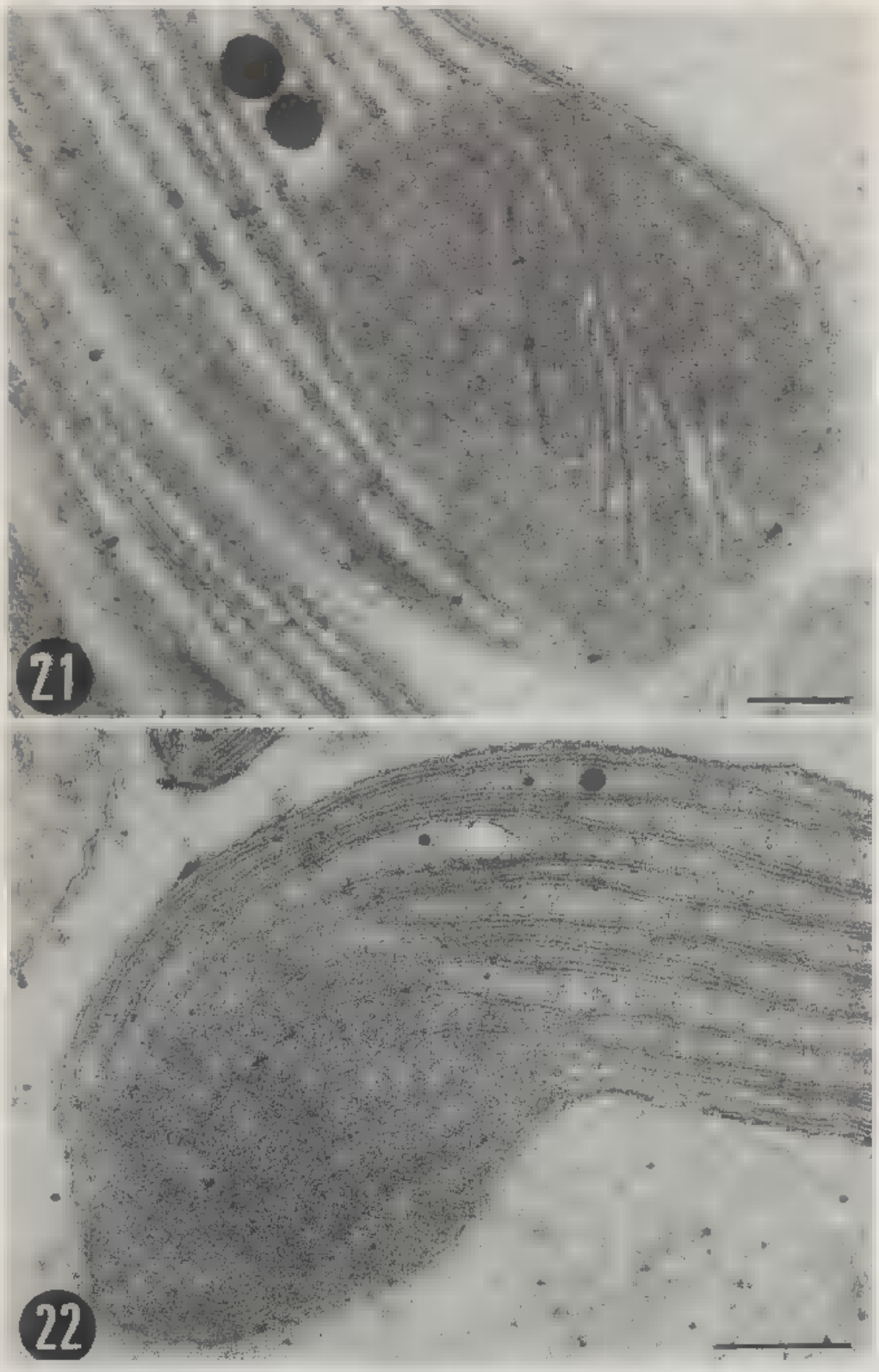
Figs 14-15. *Euglena viridis.* Portions of cells in transverse view showing peripheral chloroplasts (C) and pyrenoids (P). Insets: Higher magnification of pyrenoid regions (P). Bars = 1 μm.



Figs 16-18. *Euglena viridis.* Higher magnification of pyrenoids (P) with peripheral and traversing thylakoids. Bars = 0,5 µm.



Figs 19-20. *Euglena geniculata.* Transverse views of cells at different levels show especially the interconnected multilobed chloroplast and pyrenoid. Inset: Higher magnification of pyrenoid shown in Fig. 19. Bars = 1 μm.



Figs 21-22. *Euglena geniculata.* Higher magnification of pyrenoids showing matrices traversed by thylakoids. Bars = 0,25 µm.

SILICA-SCALED CHRYSOPHYTES IN BULGARIA

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ABSTRACT — During an examination by transmission electron microscopy of samples from 21 freshwater localities (lakes, swamps, ponds and streams) in Bulgaria, a total of 13 species of silica-scaled chrysophytes (Chrysophyceae and Synurophyceae) were identified. Only three of these species had previously been recorded from Bulgaria, and only one of these was studied by electron microscopy. Eight of the species are cosmopolitan in their distribution, three are restricted to northern temperate regions, and two have a bipolar temperate distribution. Almost all the species have been reported from adjacent countries.

RÉSUMÉ — Treize espèces de chrysophytes à écailles silicieuses (Chrysophyceae et Synurophyceae) ont été identifiées par examen, en microscopie électronique à transmission, d'échantillons de 21 localités d'eau douce (lacs, étangs, tourbières et rivières), en Bulgarie. Trois de ces espèces étaient déjà signalées en Bulgarie, mais une seule avait fait l'objet d'une étude en microscopie électronique. Huit des espèces sont cosmopolites, les cinq autres sont limitées aux régions tempérées, dont deux avec une distribution tempérée bipolaire. Presque toutes ces espèces sont connues dans des pays limitrophes.

KEY-WORDS: Bulgaria, Chrysophyceae, distribution, freshwater algae, Heterokonta, new record, Stramenopiles, Synurophyceae, transmission electron microscopy.

INTRODUCTION

The flora of silica-scaled chrysophytes (belonging to the classes Chrysophyceae and Synurophyceae) in Bulgaria is very little known (Kristiansen *et al.*, in press). In contrast, there are several more in-depth investigations from the adjacent countries of Romania (e.g. the works by Péterfi, 1966; Peterfi & Momeu, 1976; Momeu & Péterfi, 1987) and Greece (Kristiansen, 1980, 1983), and also from Hungary (Kristiansen & Padisák, 1992; Kiss & Kristiansen, 1994).

In Bulgaria, some early finds of species of *Mallomonas* and *Synura* have been reported by Valkanov (1928), with light microscopy, but such observations are no longer considered reliable. These and other LM documented finds of chrysophytes have been listed and evaluated in the survey by Kristiansen *et al.*, in press. Electron microscopy of the

silica scales is indispensable for species identification within this group of organisms (see e.g. Kristiansen, 1996), but the only EM study as yet on Bulgarian material is the description of a bloom of *Mallomonas acaroides* Perty from a pond in the Rila Mountains (Kristiansen, 1971).

MATERIALS AND METHODS

The samples were collected from the water surface in 1 l glass or plastic bottles and fixed with 2-4 % formaldehyde. Electron microscopy of the silica scales was done in the Department of Phycology, University of Copenhagen. Droplets of sedimented material from the samples were dried onto formvar/carbon coated grids, washed in distilled water and after drying the grids were examined in a JEOL JEM 100SX electron microscope.

The 33 samples were taken from 21 localities (Fig. 1) that included some lowland temperate lakes (Loc. 1, 2), lowland as well as mountain swamps (Loc. 3, 4, 5, 6, 7), mountain stream and peatbogs (Loc. 8, 9, 10), high mountain lakes (Loc. 11, 12, 13, 14, 15, 16, 17, 18, 19, 20), and a high mountain pool (Loc. 21). The localities are located at about 42-44° N.Lat. Localities are listed in Table 1.

Fig. 1. Map of Bulgaria showing the location of the investigated localities.

Table 1. List of sampled localities.

Locality 1. Lake "Beloslavsko ezero" — located west to the town of Varna at the Black Sea Coast, area of 400 ha, maximum depth 3.5 m, average depth 2.3 m, volume 9 10^6 m^3, previously freshwater, recently changed to brackish water (salinity > 5 ‰), surrounded by reeds. Sampled on 23.05.92 by Dr Delov from Sofia University (SU).

Locality 2. Srebarna Lake — located at the right bank of the Danube River, total area of 2.4 km^2 and open water area of 0.6 km^2, maximum depth at the sampling time 1.2 m, volume 3.7 10^5 m^3, pH varying between 7 and 8.5, freshwater, highly eutrophic holo-polymictic temperate lake surrounded by reeds. Sampled on 20.05.92, 6.06.92, 22.04.93, 6.07.93, 10.12.94 by Dr M. Stoyneva (SU), N. Michov, Dr M. Marinov, and Dr T. Michev from Bulgarian Academy of Sciences (BASc).

Locality 3. Swamp "Blato Tarlitza" — located at the right bank of the Danube River and adjacent to the Srebarna Lake, maximum depth at the sampling time 0.5 m, freshwater, highly eutrophic holo-polymictic temperate swamp. Sampled on 27.04.94 by Dr M. Stoyneva, N. Michov and Dr T. Michev.

Locality 4. Swamp (A) in the South Park of Sofia town — located in the central part of Sofia (about 560 m above sea level), maximum depth at the sampling time 0.8 m, freshwater holo-polymictic temperate swamp overgrown by hydrophytes. Sampled on 15.04.88 by Dr M. Stoyneva.

Locality 5. Swamp (B) in the South Park of Sofia town — located in the central part of Sofia (about 560 m above sea level), maximum depth at the sampling time 0.5 m, freshwater holo-polymictic temperate swamp surrounded by rushes. Sampled on 21.06.89 by Dr M. Stoyneva.

Locality 6. Swamp "Dolno Bojansko blato" — located in the south-western part of Sofia town at the foot-hills of Vitosha Mountain (about 610 m above sea level), maximum depth at the sampling time 0.4 m, freshwater holo-polymictic swamp with small open water areas among the rush-bed. Sampled 16.07.94 by Dr M. Stoyneva.

Locality 7. Swamp "Gorno Bojansko blato" – located in the south-western part of Sofia town at the foot-hills of Vitosha Mountain (about 620 m above sea level), maximum depth at the sampling time 1.2 m, freshwater holo-polymictic swamp with a rush-bed at its northern and *Sphagnum* at its southern part. Sampled on 16.07.94 by Dr M. Stoyneva.

Locality 8. Stream "Suhodolska reka" located in the south-western direction towards the town of Sofia, at about 580 m above sea level. Sampled on 12.06.92 by Dr M. Stoyneva, Dr St. Kovachev (SU), Dr T. Michev and Dr V. Velev (BASc).

Locality 9. Peat-bog at Vitosha Mountain — a small bog overgrown by *Sphagnum*, located at about 1000 m above sea level. Sampled on 18.07.94 by Dr M. Stoyneva.

Locality 10. Peat-bog in the Rila Mountains — a small bog located near the top of Maljovitza at about 2350 m above sea level. Sampled on 27.07.94 by Dr M. Stoyneva and Dr T. Michev.

Locality 11. Lake "Dolno Kamilsko ezero" — located in the Rila Mountains at about 2300 m above sea level. Sampled on 28.07.94 by Dr M. Stoyneva and Dr T. Michev.

Locality 12. Lake "Gorno Kamilsko ezero" – located in the Rila Mountains at about 2350 m above sea level by Dr M. Stoyneva and Dr T. Michev.

Locality 13. Lake "Strashnoto ezero" — located in the Rila Mountains at 2408 m above sea level, water surface area 0.14 ha, maximum depth 2.0 m, pH = 7.0. Sampled on 28.07.94 by Dr M. Stoyneva and Dr T. Michev.

Locality 14. Lake "Suhoto ezero" — located in the eastern part of the Rila Mountains at about 2475 m above sea level, water surface area 0.9 ha. Sampled on 19.10.94 by Dr Sp. Tonkov (SU) and Dr D. Dimitrov (SU). Locality 15. Lake 1 from the group "Urdini ezera" — located in the western part of the Rila Mountains at 2375 m above sea level, maximum depth 4.7 m, average depth 1.9 m, water surface area 0.9 ha, pH = 6.6. Sampled on 18.10.69 by Dr St. Draganov (SU).

Locality 16. Lake 2 from the group "Urdini ezera" — located in the western part of the Rila Mountains at 2278 m above sea level, maximum depth 6.6 m, average depth 3.6 m, water surface area 2.5 ha, volume 8.95 10^4 m^3, pH = 6.8. Sampled on 18.10.69 by Dr St Draganov.

Locality 17. Lake 3 from the group "Urdini ezera" — located in the north-western part of the Rila Mountains at 2339 m above sea level, maximum depth 4.7 m, average depth 2,5 m, water surface area 2,3 ha, volume 5.95 10^4 m^3, pH = 7.7. Sampled on 18.10.69 by Dr St. Draganov.

Locality 18. Lake "Okoto na Todora" — located in the northern part of the Pirin Mountains at 2062 m above sea level, water surface area 0.3 ha, maximum depth 5.5 m, average depth 2.5 m, volume 6.6 10^3 m^3. Sampled on 26.10.89 by Dr M. Stoyneva and Dr D. Lazarov.

Locality 19. Lake "Ribno ezero" — located in the northern part of the Pirin Mountains at 2190 m above sea level, water surface area 6 ha, maximum depth 12 m. Sampled on 30.06.92 by Dr V. Koicheva.

Locality 20. Lake "Banderishko ezero" — located in the northern part of the Pirin Mountains at 2312 m above [illegible] level, water surface area 0.4 ha, average depth about 1 m. Sampled on 26.10.89 by Dr M. Stoyneva and Dr D. Lazarov.

Locality 21. A pool near the tourist house "Vichren" — in the northern part of the Pirin Mountains at about 2000 m above sea level, with mass development of *Chaetophora elegans* (Roth) C. Ag. Sampled on 26.10.89 by Dr M. Stoyneva and Dr D. Lazarov.

RESULTS AND DISCUSSION

A total of 13 species were found, including four belonging to the Chrysophyceae and nine to the Synurophyceae. They have been listed, together with the localities, in Table 2, and electron micrographs of their silica structures are given as Figs 2-19. Some of the scales appeared rather corroded, affecting the quality of several of the pictures. In Fig. 12, e.g., the secondary layer of the scale has almost disappeared, but it can be better seen in the original micrographs.

Table 2. Chrysophyte taxa and their occurrence in the investigated localities

Spiniferomonas abei Takahashi: Loc. 12
Spiniferomonas trioralis Takahashi: Loc. 2, 19
Paraphysomonas takahashii Cronberg & Kristiansen: Loc. 2
Paraphysomonas vestita (Stokes) De Saedeleer: Loc. 2 (four samples)
Mallomonas matvienkoae (Matvienko) Asmund & Kristiansen var. *matvienkoae*: Loc. 12
Mallomonas papillosa Harris & Bradley var. *papillosa*: Loc. 15, 20, 21
Mallomonas caudata Ivanov *emend.* Krieger: Loc. 5, 8, 19
Mallomonas actinoloma Takahashi var. *maramuresensis* Péterfi & Momeu: Loc. 2, 12
Mallomonas alpina Ruttner *in* Pascher: Loc. 19
Mallomonas tonsurata Teiling: Loc. 5, 8, 19
Mallomonas acaroides Perty *emend.* Ivanov var. *acaroides*: Loc. 5, 8, 15, 17, 18, 19, 20, 21
Mallomonas pumilio Harris & Bradley var. *munda* Asmund: Loc. 20
Synura petersenii Korshikov: Loc. 5, 8, 19, 22

The Chrysophyceae were represented by the genera *Paraphysomonas* and *Spiniferomonas*. *Spiniferomonas trioralis* (Fig. 3) is a widely recorded cosmopolitan species. It is recorded from neighbouring Greece (Kristiansen, 1980), and also from Hungary (Kristiansen & Padisák, 1992). *Spiniferomonas abei* (Fig. 2) has scattered records from temperate and arctic regions in the northern and southern hemisphere. In Europe it has hitherto been recorded from Denmark (Kristiansen, 1978) and Norway (Skogstad, 1986). The find in Bulgaria thus represents its southernmost reported European occurrence. *Paraphysomons vestita* (Fig. 5) is one of the world's most widely distributed chrysophytes, found in all parts of the world. It has previously both been recorded from Greece (Kristiansen, 1980) and Hungary (Hajdu, 1975; Kristiansen & Padisák, 1992). *Paraphysomonas takahashii* (Fig. 4) has a much more scattered occurrence. Since its description from Sweden (Cronberg & Kristiansen, 1980) it has been found in several localities in northern temperate regions, in Asia, America and Europe. The European finds further include Iceland (Kristiansen, 1995), Denmark (Thomsen *et al.*, 1981), and Finland (Hällfors & Hällfors, 1988). Thus, it has never been recorded so far south as in Bulgaria before.

The Synurophyceae were represented by the two genera *Mallomonas* and *Synura*, with eight and one species, respectively. *Mallomonas matvienkoae* f. *matvienkoae* (Fig. 6) is a cosmopolitan taxon, occurring mainly in temperate and subtropical regions. It has also been recorded from Romania (Asmund & Kristiansen, 1986; Péterfi & Momeu, 1977), whereas another variety, var. *grandis* Dürrschmidt & Cronberg, has its main occurrence in the tropics (Cronberg, 1989; Wujek & Saha, 1996). *Mallomonas papillosa* var. *papillosa* (Fig. 10) is a widely distributed cosmopolitan taxon, also found in many European countries. It was recorded from Romania by Péterfi & Momeu (1976). *Mallomonas caudata* (Figs 8-9) has previously been recorded from the Bulgarian Danube section by LM (Szemes, 1967) and it is one of the few species which can be reliably identified in this way. It is an almost cosmopolitan species, and it has been recorded from the adjacent countries of Romania and Greece (Asmund & Kristiansen, 1986). *Mallomonas actinoloma* var. *maramuresensis* (Fig. 7) was described by Péterfi & Momeu (1976) from the Maramures Mountains in Romania. Later it has been shown to have a wide but scattered northern distribution, in Northern Europe and Japan (see Asmund & Kristiansen, 1986), but it has not been recorded from adjacent countries other than Romania. *Mallomonas alpina* (Fig.11) is a common cosmopolitan species. It has also been found in in the adjacent countries Romania and Greece, and also in Hungary (Asmund & Kristiansen, 1986; Kristiansen & Padisák, 1992). *Mallomonas tonsurata* (Figs 12-13) is one of the world's most widely distributed species. It has also been found in the neighbouring Greece and in Hungary (Kristiansen, 1980; Kristiansen & Padisák, 1992), but has apparently never been recorded from Romania. The species has previously been identified by LM on dried material collected in Bulgaria by Valchanova (1995) and by Stoyneva & Valchanova (1997). *Mallomonas acaroides* (Figs 14-17) was found in several localities. Specimens found in localities 5, 18 and 19 had scales with a very weakly developed ornamentation, whereas the scales in the other samples had a well developed reticulum on the shield. Most often both serrated bristles and helmet bristles are present, but in some localities only serrated bristles were found, in other localities only helmet bristles (Loc. 15, 16, 18, 21). The previous find of a population of this species in Bulgaria examined by EM showed all bristles developed as helmet bristles (Kristiansen, 1971). Fott (1962) distinguished four varieties of *M. acaroides* based on bristle characters, but the three of them, excluding the very deviating var.*inermis* Fott, should be united as *M. acaroides* var. *acaroides*, with a variable ratio between helmet bristles and serrated bristles (see Asmund & Kristiansen, 1986). It is not known whether bristle morphology in this species depends on temperature,

such as is the case in *M. crassisquama* (Asmund) Fott (see Siver & Skogstad, 1988). *M. acaroides* has previously been recorded from Bulgaria by LM by Valkanov (1928); the identification was later confirmed by EM (Kristiansen, 1971). Further LM identifications include records by Valkanov (1934), Vodenicharov (1962), Szemes (1967), Valchanova (1995) and Stoyneva & Valchanova (1997). It is widely distributed in the northern temperate and subarctic regions, and it has also been recorded from Romania and Hungary (Asmund & Kristiansen, 1986; Kiss & Kristiansen, 1994). *Mallomonas pumilio* var. *munda* (Fig. 18, only this single scale found) is distributed in temperate regions. It is widely recorded from Europe, and it has also been found in Canada and in temperate parts of S. America. In adjacent countries it has also been found in Romania (Momeu & Péterfi, 1987).

Only one species of the genus *Synura* has been found, viz. *Synura petersenii* (Fig.19). It has also been found in the adjacent countries: Romania (Péterfi, 1966), Greece (Kristiansen, 1980), Hungary (Kristiansen & Padisák, 1992) and it is one of the world's most widely distributed chrysophyte. Another species has been recorded as frequent in Bulgaria, viz. *Synura uvella* Ehr. (Valkanov, 1926, 1928; Vodenicharov, 1962, 1967; Naidenov, 1964; Naidenov & Saiz, 1977; Saiz, 1978; Stoyneva, 1991; Valchanova, 1995), but without examination of the silica-scale structure. *"Synura uvella"* was previously used as a common name for any species of the genus, and thus it cannot be seen which species is actually referred to in these works (Kristiansen *et al.*, in press).

As regards the occurrence of the species in the examined localities, the following remarks can be made. There were no species reported from the acid peat bogs (localities 9,10). The following species were confined to the mountain lakes (localities 12-21):*Spiniferomonas abei*, *Mallomonas matvienkoae*, *M. papillosa*, *M. alpina*, and *M. pumilio* var. *munda*. This is in accordance with their general preference for somewhat oligotrophic conditions. *Paraphysomonas takahashii* and *P. vestita* were only found in the highly eutrophic lake loc.2, as is often seen in this heterotrophic genus. The remaining species were found in a variety of locality types, reflecting that most of them, especially *Synura petersenii*, have a wide environmental range.

It is seen from the above that altogether 13 species were found during this investigation, only one of which has been recorded previously from Bulgaria by EM examinations, and two other from LM observations only. More species could certainly be found, and indeed several other species have been recorded from LM observations (Kristiansen *et al.*, in press). In the adjacent countries many more species of silica scaled chrysophytes have been found, e.g. in Greece 38 species of silica-scaled chrysophytes have been identified by EM (Kristiansen, 1980, 1983).

Most of the species are widely distributed and more or less cosmopolitan, except *Mallomonas acaroides*, *Mallomonas actinoloma* var. *maramuresensis* and *Paraphysomonas takahashii* which are restricted to the Northern Hemisphere, the two latter having very scattered occurrences. *Spiniferomonas abei* and *M. pumilio* var. *munda* have bipolar temperate occurrences (see Kristiansen & Vigna, 1996).

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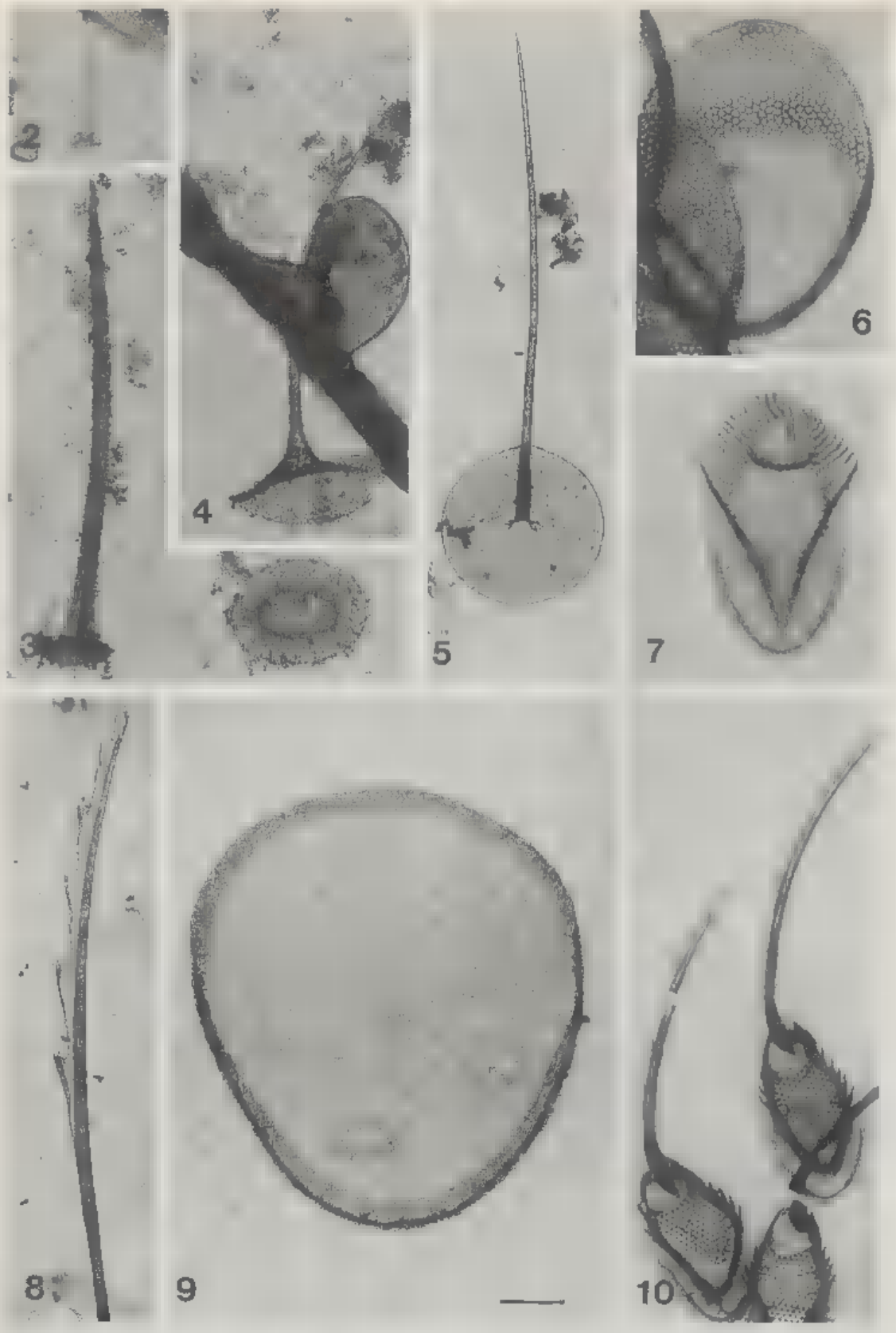
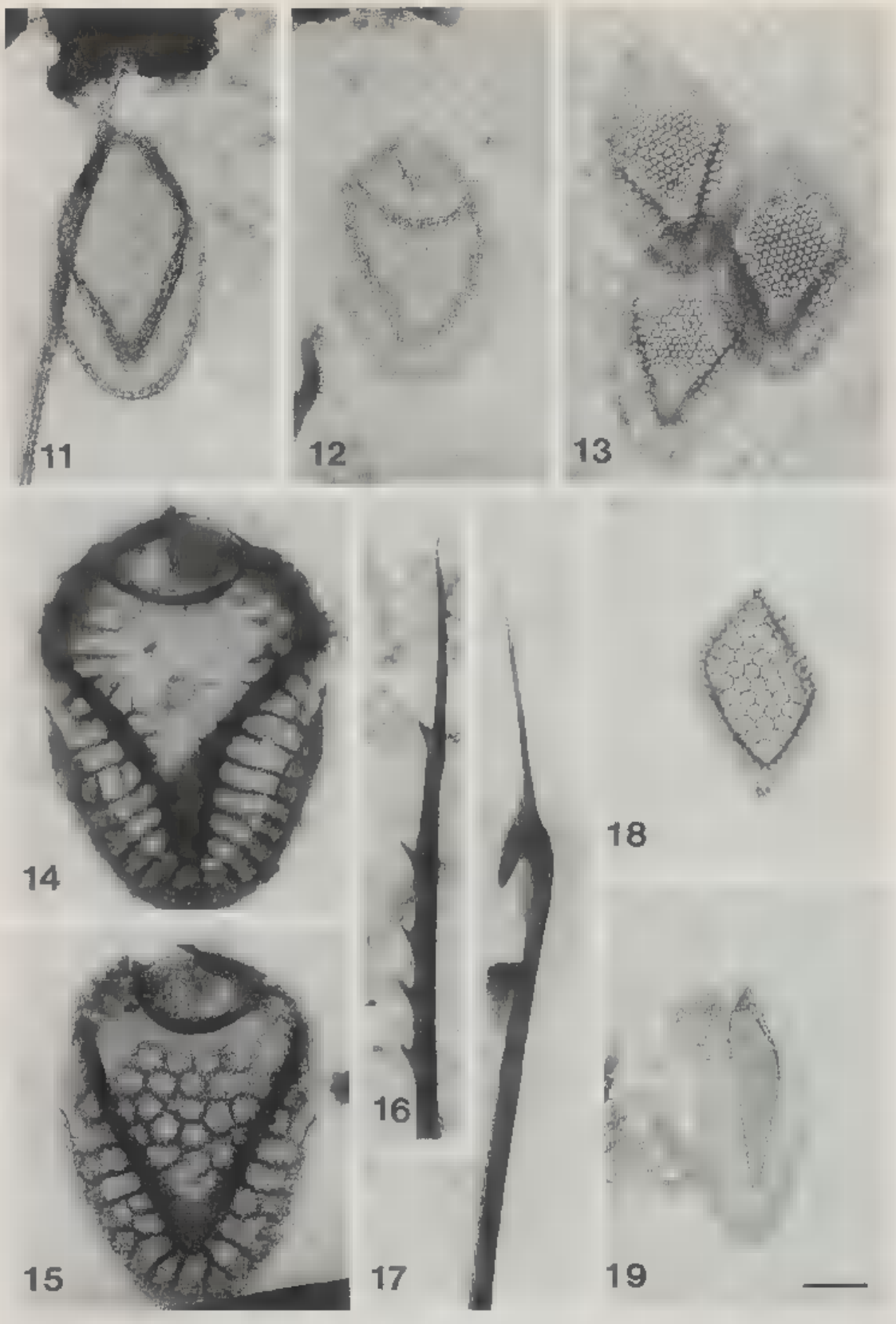


Figs 2-10. Fig. 2. *Spiniferomonas abei*, spine scale (12). Fig. 3. *S. trioralis*, spine scale (2) and plate scale (19). Fig. 4. *Paraphysomonas takahashii*, spine scale (2). Fig. 5. *P. vestita*, spine scale (2). Fig. 6. *Mallomonas matvienkoae* f. *matvienkoae*, scale (12). Fig. 7. *M. actinoloma* var. *maramuresensis*, scale (19). Figs 8-9. *M. caudata*, bristle and scale (19). Fig. 10. *M. papillosa* f. *papillosa*, scales with bristles (21). Scale bar indicates 1 µm, except in Fig. 8, 10 µm. Locality numbers indicated.



Figs 11-19. Fig.11. *Mallomonas alpina*, domeless body scale (19). Figs 12-13. *M. tonsurata*, apical and body scales (19 and 8). Figs 14-15. *M. acaroides* var. *acaroides*, body scales (16 and 18). Figs 16-17. *M. acaroides* var.*acaroides*, serrated bristle (18) and helmet bristle (20). Fig. 18. *M. pumilio* var. *munda*, body scale (20). Fig. 19. *Synura petersenii*, posterior body scale (19). Scale bar 1 μm. Locality numbers indicated.

NANOFLAGELLATES OF EAST PACIFIC COASTAL WATERS: MORPHOLOGY, TAXONOMY, AND BIOGEOGRAPHY OF WEAKLY CALCIFIED COCCOLITHOPHORIDS (PRYMNESIOPHYCEAE)

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ABSTRACT — A transmission electronmicroscopical examination of whole mounts of cells prepared from samples collected off central California, September 1989, from the Sea of Cortez, (Mexico), January 1990, and from San Juan Islands, Washington, August 1983, has led to the identification of 7 taxa of weakly calcified nanoflagellates, the majority of which are related to forms which have their main distribution in polar regions.

RÉSUMÉ — Un examen, en microscopie électronique à transmission, de cellules entières préparées à partir d'échantillons prélevés au large de la Californie centrale, en septembre 1989, en mer de Cortez (Mexique), en janvier 1990, et aux îles San Juan, Washington, en août 1983, a conduit à l'identification de nanoflagellés faiblement calcifiés dont la majorité est apparentée à des formes distribuées principalement dans les régions polaires.

KEY WORDS: biogeography, central California waters, Coccolithophorids, micro-algae, morphology, nanoflagellates, Prymnesiophyceae, San Juan Islands, Sea of Cortez.

INTRODUCTION

Coccolithophorids are nanoflagellates (< 20 µm) that have calcium carbonate coccoliths located externally as a cellular periplast. Coccoliths may be robust and have complex crystallographic structure, or may be more weakly calcified and possess a relatively simple structure. Due to the refractive nature of robust coccoliths they are frequent objects of study and are useful as markers of oceanic water masses and climate change. Coccolithophorids have been shown to be more diverse and abundant with

increasing temperature and they have been considered to be absent at temperatures below 2° C. Whilst this has generally been found to be true, a series of papers by Manton and co-workers (Manton & Oates, 1975 *et seq.*) and Thomsen and co-workers (Thomsen, 1980a *et seq.*) have demonstrated the presence of a polar community of nanoplanktonic and weakly calcified coccolithophorids that thrive at temperatures as low as -2° C. In recent papers (Thomsen, 1986; Garrison & Thomsen, 1993; Marchant & Thomsen, 1994) it has been reported that the cold water coccolithophorid community consists exclusively of non-photosynthetic forms.

During September 1989 we had the opportunity to participate in an oceanographic cruise in the waters off central California allowing us to contribute new data on the biogeography, morphology and taxonomy of marine coccolithophorids from genera which are otherwise mostly associated with polar regions (Thomsen, 1981; Thomsen *et al.*, 1988). This is the third paper in a series of reports on the biodiversity of nanoflagellates in central Californian waters (see also Thomsen *et al.*, 1991a; Thomsen & Buck, 1998). This paper additionally includes material from other localities on the fringes of the Pacific Ocean, viz. San Juan Island, Washington, and the Sea of Cortez, Mexico.

The present paper reports on the finding of specimens identical to or related to members of the polar community at temperate and sub-tropical Pacific Oceanic sites. Recently documented life-histories of polar coccolithophorids combine e.g. species from the genus *Papposphaera* with species from the genus *Turrisphaera* (Thomsen *et al.*, 1991b). Due to this uncertainty we will refrain from formally describing new taxa, with the exception of *P. bourrellii* sp. nov.

MATERIALS AND METHODS

Water samples were collected from positions off California (Fig. 1) during the R/V "Point Sur" primary productivity cruise number 8 (PP8), September 25-30, 1989, and here the origin (station number) of material selected for publication is indicated in the legends. A 5 liters Niskin bottle was used to obtain the surface samples. Deeper samples were additionally collected from some stations. The nanoflagellates were, in most cases, concentrated by means of centrifugation of approx. 200 ml of prefiltered (mesh size 20 µm) seawater from each station. Light and electron microscopical whole mounts were made according to established procedures (Moestrup & Thomsen, 1980; Thomsen, 1982). Whole mounts for TEM were shadowcast with chromium. The microscope used was a JEOL 100B at the electron microscopical facility at University of California at Santa Cruz.

Surface water samples from the San Juan Islands, Washington, were collected in August 1983, at localities in the vicinity of Friday Harbor, Turn Island, Knob Island and McConnell Island (approx. 123° W, 48.5° N). The protocol was similar to that described above, with the exception that the prefiltering screen used had a mesh size of 40 µm. The whole mounts were shadowcast with gold-palladium and examined on a JEOL T7 electron microscope at the Botanical Institute, University of Copenhagen.

Samples from the Sea of Cortez, were collected at Bahia de Los Angeles, Mexico (*ca* 29° N, 113.5° W) during January 1990. The water samples collected were processed in the trunk of a rental car, using ■ sequential filtration on 3 µm Millipore filters followed by centrifugation of prefiltered (20 µm) surface water. The whole mounts were shadowcast with chromium and examined on a JEOL 100S electron microscope at the Botanical Institute, University of Copenhagen.

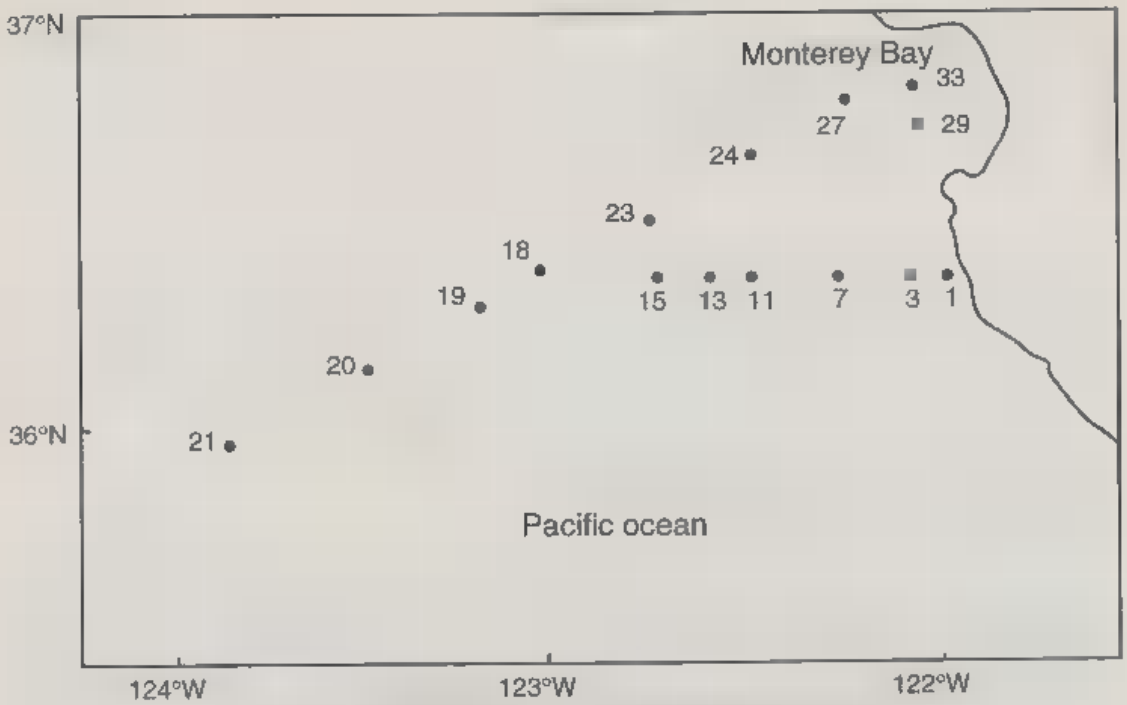

Fig. 1. Station map from central California waters.

OBSERVATIONS

In describing the taxa we follow taxonomic recommendations as suggested by Jordan *et al.* (1993), and the classification of extant Haptophyta as proposed by Jordan & Green (1994). The terminology used is in accordance with Jordan *et al.* (1995).

Class Prymnesiophyceae
Order Prymnesiales
Family Pappospharaceae

***Papposphaera* Tangen**

This genus possesses pappoliths of one type only. A "pappolith" (Norris, 1983) is a heterococcolith with a calcified rim in the form of a narrow, slightly flaring wall composed of elements of two alternating types (Jordan *et al.*, 1995). The base plate calcification is weakly developed and variable among the species presently allocated to the genus. A central process is often present. Thomsen *et al.* (1991b) showed that species of *Papposphaera* and species of *Turrisphaera* are life history stages of a single organism. This conclusion was based on the observation of single, naturally occurring cells possessing both the pappoliths, and turriform holococcoliths characteristic of *Turrisphaera*. The genus *Papposphaera* at present comprises the type species *P. lepida* Tangen ("*Turris-*

phaera" phase unknown), *P. arctica* (Manton *et al.*) Thomsen *et al.* (syn. *Pappsphaera sarion* Thomsen); *P. borealis* (Manton *et al.*) Thomsen *et al.* (syn. *Pappsphaera sagittifera* Manton *et al.*), *P. obpyramidalis* Thomsen ("*Turrisphaera*" phase unknown), *P. polybotrys* (Thomsen) Thomsen *et al.* ("*Pappsphaera*" phase unknown), *P. simplicissima* Thomsen ("*Turrisphaera*" phase unknown), *P. thomsenii* Norris ("*Turrisphaera*" phase unknown) (Manton *et al.*, 1976a,b; Norris, 1983; Tangen, 1972; Thomsen, 1980b; Thomsen *et al.*, 1988, 1991b).

Species of "*Turrisphaera*" have proved very difficult to identify from TEM whole mounts, and it has been obvious that a reliable discrimination of taxa is often not possible, unless supported by the simultaneous discovery (e.g. as combination cells) of the *Pappsphaera* counterpart in the *Turrisphaera-Pappsphaera* life history. The present material did not comprise any combination cells, which is one reason why we have tentatively to group the "*Turrisphaera*"-cells encountered under provisional headings.

***Pappsphaera lepida* Tangen, Figs 2-8.**
(description of "*Pappsphaera*" phase)

The original description of this species (Tangen, 1972) was based on light and scanning electron microscopy. The cell body diameter was 4.5-7 µm, whereas the diameter of the coccosphere ranged from 11-16 µm. Two flagella, 2-3 times the diameter of the coccosphere, but no haptonema, were seen in the type material. Tangen (1972) cautiously stated that the genus is "apparently" characterised by having 2 chloroplasts. The coccoliths (50-100 per cell) are all of the same basic type with an elliptical to subcircular base plate (long axis: 0.8-1.4 µm) and a central process (2-3.9 µm long) which terminates in a wide, subcircular to four-lobate, funnel-shaped structure (diameter 1.1-2.1 µm). Four decurrent ridges from the central process form a distinct, calcified cross on the base plate. Limited by the resolution of the SEM Tangen (1972) was only able to describe the scale rim (0.2-0.35 µm high) as consisting of 18-24 thin plate elements (pentagonal) and with an irregularly toothed upper rim.

Transmission electron micrographs of South African material published by Manton & Oates (1975, figs 3-4) added significant details to the first description of *P. lepida*. A second ring of narrow, rod-shaped crystallites was found to be part of the base plate rim. An additional feature clearly resolved in the transmission electron microscope was the radiating ridges of the organic, subtending scale. More recently *P. lepida* (as *Pappsphaera* sp.) from the North Pacific Central Gyre was also described and illustrated (TEM) (Hoepffner & Haas, 1990). These authors hesitated to identify the material examined as *P. lepida* because the protoplast comprised coccoliths of different sizes, though apparently with the same overall morphology, and also because of the finding of special structural features interlocking the process shaft and the distal plates. Other species of *Pappsphaera*, viz. *P. borealis* (syn. *P. sagittifera*), have been found to be characterised by a pronounced polarity in the development of the single type of coccolith present. This variability appears to be mostly in size, with more fully developed and larger coccoliths typically clustering at the apical pole of the cell. We also believe that *P. lepida* sometimes shows a similar gradation in coccolith size from one end of the cell to the other, and that this is in fact what Hoepffner & Haas (1990) observed. The interlocking, structural details, e.g. a small wristlet-like expansion of the distal part of the process shaft, and a displacement (eccentricity) of the individual quadrants to produce a small central and angular hole that matches the "wristlet", was also illustrated but not commented on by Manton &

Oates (1975). It was obviously beyond the resolution power of the SEM used by Tangen (1972) when examining the type-material. In our opinion the North Pacific Gyre material examined by Hoepffner & Haas (1990) can be unequivocally identified as *P. lepida*.

The Californian specimen (Figs 2-4) is similar to previously examined material (Tangen, 1972; Manton & Oates, 1975; Hoepffner & Haas, 1990), and shares with the cells from the North Pacific Gyre a pronounced polarity in development of the individual coccoliths. There is a gradual decrease in distal quadrant edge length from the apical pole (2.2 µm) towards the antapical pole (1.3 µm). Details of the interlocking devices, *i.e.* a wristlet-like, cross-shaped termination of the central process, and slightly offset quadrants producing a matching cavity (Fig. 4) are virtually indistinguishable from those illustrated by Hoepffner & Haas (1990). The distal appendage is subquadrangular on apical pole coccoliths (Fig. 3, arrow) and with rounded corners, while approximately circular on coccoliths at the antapical cell end (Fig. 3, arrowhead).

Cells from Mexico (Figs 5-8) initially appear sufficiently different from material cited above to warrant description as an independent species. However, despite the conspicuous difference in shape of the distal appendage (perfect squares in Mexican cells) the presence within the very same periplast of a single coccolith (Fig. 8, arrowhead) with a quasi-discoid appendage, indistinguishable from e.g. those on Californian cells (Fig. 3), suggests that a species distinction cannot be based on this particular feature. It should be emphasised that the pappoliths on the Mexican cells in all other aspects (viz. scale rim and base plate calcification (Fig. 7, arrow), interlocking (Fig. 6) of shaft and distal appendage) are virtually indistinguishable from *P. lepida* pappoliths studied previously. Also, the Mexican cells (Figs 5, 8) display a pronounced reduction in size (see Fig. 3) from the apical pole (edge of distal quadrant: 1.5 µm; length of shaft: 2 µm) towards the antapical pole (edge of distal quadrant: 1 µm; length of shaft: 1 µm). A haptonema (Fig. 5, arrow) has not previously been observed in specimens identified as *P. lepida*.

BIOGEOGRAPHY: *Papposphaera lepida* is infrequently recorded. However, it is widely distributed with a concomitantly wide temperature range, indicating that this taxon is eurythermic. It appears to thrive in sea water of oceanic salinity and is thus best characterised as being stenopolyhaline. Abundance estimates based on cell enumerations of preserved material examined with the inverted microscope (Tangen, 1972) or SEM (Samtleben & Schröder, 1992) were 10^3 and 3×10^2 cells l^{-1} respectively. Previous findings: Nordåsvatnet (type locality), Western Norway (Tangen, 1972; salinity mostly > 27 PSU; temperature 6.5-20.5° C); Caribbean Sea (Throndsen, 1972; as Coccolithophorid sp. 2; see Tangen, 1972); North-West Africa (Heimdal, pers. com. in Tangen, 1972); Cape Town (Manton & Oates, 1975; 9-10° C); North Atlantic (Okada & McIntyre, 1977; St. "Charlie"); Iceland Plateau (St. 9) in the proximity of Jan Mayen (Samtleben & Schröder, 1992; approx. 2-6° C); Denmark (Thomsen, unpublished results; January 1976; 2.7° C, 26.3 PSU; June 1976; 6.3° C, 31.4 PSU (15 metres depth); Phuket, Thailand (Thomsen, unpublished results; August 1981; 27° C, 35 PSU).

***Papposphaera bourrellii* sp. nov., Figs 9-16.**
(description of "*Papposphaera*" phase)

DIAGNOSIS: The cell is spherical, *ca* 4 µm in diameter, with 2 almost equally long flagella (*ca* 20 µm) and a significantly shorter, coiling haptonema (Fig. 13, arrow). Diam. of coccosphere *ca* 13 µm. Coccoliths of one type (pappoliths). The elliptical base plate

(0.8 × 0.5 µm) is calcified along the rim and in a cruciform pattern on the scale surface (Fig. 14). The rim calcification consists of 2 series of crystallites (Fig. 14), *i.e.* small rod-shaped crystallites (0.15 × 0.03 µm), and larger polygonal elements (ca 0.15 µm) that form the upright, irregularly toothed rim. The shaft of the central process measures ca 3.5 µm in length and supports a distal, calyx-like appendage, consisting of 4 rhomboidal, sepal-like structures (Fig. 15). Each of these measure ca 0.6 × 0.3 µm. The interconnection between the shaft and the "calyx" is facilitated by a wristlet-like distal thickening of the shaft (Fig. 15, arrow). The "*Turrisphaera*"-phase of this taxon is presently unknown.

Holotype specimen (iconotype) and type locality: Fig. 13 from San Juan Islands (McConnell Island; < 40 metres depth), Washington, USA, 8 August 1983.

Cellula sphaerica, circa 4 µm diametro, flagellis binis, subaequalibus et haptonemate manifeste breviori instructa. Diametrum coccosphaerae circa 13 µm. Coccilithi monomorphi (pappolithi). Lamina basalis elliptica secus marginem et in superficie squamae cruciforme calcificata. Calcificatio marginis e seriebus duabus crystallitarum, i.e. crystallitis parvis bacilliformibus et elementis amplioribus polygonalibus, marginem erectam irregulariter dentatam formantibus composita. Scapus processus centralis circa 3.5 µm longus, appendicem calyciformem distalem, e 4 compaginibus rhomboideis, sepaloideis compositam sustineus. Compagines circa 0.6 × 0.3 µm metientes. Junctura scapi et calycis crassitie annulari scapi distingitur. Phasis Turrisphaera hujus speciei ignota.

This species is named in honour of our late colleague, Prof. Dr Pierre Bourrelly, Muséum National d'Histoire Naturelle, Paris, who has for decades remained a key figure in research related to Chrysophyta *sensu lato*.

Pappasphaera bourrellii sp. nov. is easily distinguished from all previously described species of *Pappasphaera*, by the appearance of the calyx-like distal appendage. Another morphological characteristic which may be unique to this taxon relates to the calcification of the pappolith base-plates. The cross-shaped calcification, also known from the type-species *P. lepida*, and e.g. *P. arctica* (syn. *P. sarion*) and *P. obpyramidalis*, is also clearly present in *P. bourrellii* (Fig. 14). However, it additionally appears from Fig. 14 that in this species a major part of the base-plate surface area is covered by calcified, thin, rectangular plates. This fact has to be verified through the study of additional and better oriented coccoliths than those presently available.

In addition to the type locality, we have also found *P. bourrellii* in samples from California (Figs 9-12) and Mexico (Fig. 16; identification based on LM observations only). The Californian material is indistinguishable from the McConnell Island type material with the possible exception of the apparent lack of the extended base-plate calcification (compare Fig. 10 and Fig. 14).

This taxon has been previously illustrated (as *Pappasphaera* sp.) in Thomsen *et al.* (1994, figs 2-3).

***Pappasphaera* sp. 1, Fig. 17.**
Description of "*Pappasphaera* phase"

A fragment of a protoplast (Fig. 17) with coccoliths (pappoliths) unmistakably reminiscent of those of *Pappasphaera* spp. was encountered in a Mexican sample. The central process is elaborate and clearly consists of 4 parallel lines of crystallites that

continue in a cruciform pattern across the base-plate towards the rim of the coccolith. Large angular crystallites from the scale rim calcification are visible in Fig. 17 (arrow). The distal appendage consists of 4 quasi-rectangular, diverging plates. This material obviously represents a new species of *Papposphaera*. The formal taxonomic description is postponed until complete cells become available.

***Papposphaera* sp. 2, Figs 18-22.**
Description of "*Turrisphaera* phase"

This species possesses slender and cylindrical, turriform coccoliths which are each constructed from numerous, reticulately arranged hexagonal plates with a diameter of approximately 0.1 µm (Fig. 19). The cell (Fig. 22) has 2 almost equally long flagella (approx. 22.5 and 25 µm) and a somewhat shorter haptonema (approx. 15 µm). In some cells (e.g. Fig. 18 from Mexico and probably Fig. 22 from California) the coccoliths are relatively symmetrical structures, slightly swollen towards the distal tip, and often with some difference in overall size (1-2 µm) from one end of the cell to the other. In other cells from the same two localities (Figs 20-21) the coccoliths loose their overall symmetry and become finger-like structures. Non-mineralised, organic underlayer scales are visible in Fig. 21 (arrow). In this particular cell, part of the calcified periplast has broken away, thus exposing the underlayer of scales. At high magnification they are oval (0.5 × 0.4 µm), and with a distinct rim and a little pronounced surface pattern of ridges.

This taxon was previously illustrated (as *Turrisphaera* sp.) in Thomsen *et al.* (1994, fig. 5).

***Papposphaera* sp. 3. Figs 23-26**
Description of "*Turrisphaera* phase"

We identified a second, distinct species of "*Turrisphaera*" in the Californian material (Figs 23-26). In these organisms there is a significant difference between apical pole coccoliths and those found elsewhere in the periplast. The latter are fairly short (1.5 µm long) and symmetrical, turriform coccoliths with a conspicuous median constriction (Fig. 24, arrow). The apical pole coccoliths (3.0 µm long) are characterised by a conspicuous, unilateral proliferation of the distal end of each coccolith. Details of the proximal parts of apical pole coccoliths cannot at present be accounted for. The flagella (Fig. 26) are almost equally long (*ca* 25 µm) and the haptonema (Figs 24, 26) significantly shorter (*ca* 15 µm). Typical cell dimensions are 5-6 µm.

This species most closely resembles the "*Turrisphaera*" phase of *P. polybotrys* (Thomsen, 1980b), which is similarly characterised by two types of coccoliths of which those clustering at the apical pole have conspicuous unilateral proliferations. The main differences relate to the overall shape of the coccoliths. The apical pole coccoliths of *P. polybotrys* are elongate and narrower than those seen in the Californian material. The goblet-shaped coccoliths of *P. polybotrys* also differ in small details, e.g. with regard to the median constriction of each coccolith. It is possible that the West Greenland type material of *P. polybotrys* and the Californian material do represent the very same taxon, but, the final proof of conspecificity must come from a comparison of the *Papposphaera* phases of these populations, and thus awaits the finding of combination cells. A combination cell involving *Turrisphaera polybotrys* and *Papposphaera* sp. has been encountered in West

Greenland waters (Thomsen *et al.*, 1991b). However, due to the preservation stage of the material and the orientation of the pappoliths it was not possible to verify the identity of the *Papposphaera* counterpart of the "*Turrisphaera*" phase of *P. polybotrys*. Hoepffner & Haas (1990) found a *Turrisphaera* sp. in samples from the North Pacific central gyre which shares with the Californian material examined here the possession of apical pole coccoliths with distinctive, unilateral, distal proliferations of the turriform coccolith. The single cell from the central gyre (Hoepffner & Haas, 1990) was compared with two forms of "*Turrisphaera*" (viz. *P. borealis* and *P. arctica*) described by Manton *et al.* (1976b), but is in fact more closely reminiscent of *P. polybotrys* than the cells illustrated from Californian waters and discussed above.

Pappomonas Manton & Oates

The *Pappomonas* coccosphere is composed of more than one type of coccolith, each distributed in a specific area of the coccosphere. All coccoliths are pappoliths. The main differences between coccoliths in different parts of the periplast relate to: 1) the shape of the base-plate (circular in apical pole coccoliths) and oval in all other coccoliths, 2) the base-plate calcification (cruciform in apical pole coccoliths and in others consisting of rectangular bars of crystallites arranged roughly parallel to the long axis of the plate), and 3) the presence or absence of a central process. Coccoliths at the apical pole always carry a very conspicuous central processes. Coccoliths at the antapical pole sometimes possess somewhat shorter and also morphologically simpler central processes. The genus at present comprises 4 taxa, viz. *P. flabellifera* Manton & Oates, 1975 var. *flabellifera*; *P. flabellifera* var. *borealis* Manton et al., 1976; *P. virgulosa* Manton & Sutherland, 1975; and *P. weddellensis* Thomsen *in* Thomsen *et al.*, 1988. It was reported by Thomsen *et al.* (1991b), that species of *Pappomonas* and species of *Trigonaspis* Thomsen (Thomsen, 1980a) sometimes form combination cells, which indicates that the taxa involved (*P. flabellifera* var. *borealis* and *Trigonaspis* cf. *diskoensis* Thomsen, 1980) are different phases of the same life-cycle. The genus *Trigonaspis* Thomsen, 1980, is closely related to *Turrisphaera*, the main difference being the shape of the crystallites, which are triangular in *Trigonaspis*. This genus currently comprises three species, viz. *T. diskoensis* Thomsen, 1980; *T. minutissima* Thomsen, 1980; and *T. melvillea* Thomsen *in* Thomsen *et al.*, 1988. The genus *Pappomonas* takes priority over *Trigonaspis*. However, the formal new combination of taxa should be postponed until more examples of *Pappomonas/Trigonaspis* combination cells, in which both phases can be unequivocally identified, have been documented. Preliminary results (Østergaard, 1993) indicate that *P. virgulosa* form combination cells with *Balaniger balticus* Thomsen & Oates (Thomsen & Oates, 1978).

Pappomonas flabellifera Manton & Oates var. *flabellifera*. Figs 27-29

This taxon was found both in Californian (Figs 28-29) and Mexican water samples (Fig. 27). In all cases the cells were found to be in complete morphological and dimensional agreement with previously examined material. Antapical cell end pappoliths sometimes possessed reduced and morphologically simplified central processes (Figs 27-28). The micrograph (Fig. 29) has been previously published by Thomsen *et al.* (1994, fig. 9).

BIOGEOGRAPHY: *P. flabellifera* var. *flabellifera* is currently known from South Africa (Manton & Oates, 1975), West Greenland (Thomsen, 1981), Denmark (Thomsen, unpublished results). California and Mexico.

Genera *incertae sedis*

***Polycrater* Manton ■ Oates, 1980.**

This genus is unusual not only because of a unique morphology of the individual coccolith, but also in having a microcrystalline substructure based on calcium carbonate as aragonite instead of the more usual calcite (Manton & Oates, 1980). A coccolith consists of 4 petal-like segments (Fig. 31; arrows) forming a bowl, which on the convex side supports a heavily calcified and cruciform, lobed structure (Fig. 31; arrowheads) in which each arm is lined up exactly underneath the bordering lines between the petal-like segments. Contrary to other genera considered, which have their main distribution within temperate and polar regions, the genus *Polycrater* is apparently restricted to sub-tropical and tropical regions.

***Polycrater galapagensis* Manton & Oates, 1980, Figs 30-31.**

The Californian material exactly matches the type specimen from the Galapagos Islands (Manton & Oates, 1980). The cells illustrated here also document the presence of a haptonema (Fig. 30; arrow). When completely stretched out the haptonema is approximately half the length of the flagella. The micrograph (Fig. 30) has been previously published in Thomsen *et al.* (1994, fig. 6).

BIOGEOGRAPHY:*P. galapagensis* is recorded from the Galapagos Islands (Manton & Oates, 1980; 22° C and oceanic salinity), the Atlantic Ocean (Chrétiennot-Dinet, 1990), the Mediterranean Sea, Egypt (Thomsen, unpublished results), California and Mexico. This taxon appears to have a world-wide distribution limited to sub-tropical and tropical regions.

DISCUSSION

Due to past sampling being biased towards coastal and polar regions, the lightly calcified genera (e.g. *Papposphaera*, *Pappomonas*, *Turrisphaera*, *Trigonaspis*, *Wigwamma*) that need to be studied as TEM whole mounts for identification purposes, are thought to be found mainly in regions of the world oceans that border the main biogeographical provinces of coccolithophorid distribution. The present paper documents that these particular genera are also present in regions where typical coccolithophorids are abundant.

The polar representatives of the lightly calcified coccolithophorid genera have recently been shown to be aplastidic (Garrison & Thomsen, 1993; Marchant & Thomsen, 1994). This was deduced through a combination of epifluorescence and phase contrast

microscopy of recently collected specimens viewed as whole mounts for light microscopy prepared according to the procedure described by Thomsen (1982). Whether the same genera are also aplastidic when found outside regions characterised by prolonged periods of darkness needs, as a minimum requirement, to be verified from microscopy of freshly collected material in which the chlorophyll autofluorescence has not yet faded. Examination of thin sections with TEM for the presence (absence) of chloroplasts and/or food vacuoles would unequivocally both validate these earlier findings and also give a first hint on whether the physiology of these organisms is apt to change with latitude.

Considering the existence of complex life histories that link morphologically dissimilar taxa (viz. *Papposphaera-Turrisphaera*, *Pappomonas-Trigonaspis*; see Thomsen *et al.*, 1991b), it is interesting to note that the majority of the cells collected from Californian and Mexican waters can be segregated into 2 species of *Papposphaera* (*P. lepida* and *P. bourrellii*) and 2 species of "*Turrisphaera*" (*Papposphaera* sp. 2 and 3). It is, despite the present lack of combination cells, tempting to hypothesise that these 4 forms will eventually be found to represent 2 species, that for yet unknown reasons, can switch between a *Papposphaera* (heterococcolithophorid) phase and a *Turrisphaera* (holococcolithophorid) phase, a switch that is most likely linked to changes in ploidy level (Billard, 1994).

The haptophytes are important contributors to biogeochemical cycles in marine environments. They are intimately associated with sulphur and carbon fluxes, both of which are instrumental in global climate change scenarios. The importance of the organisms we describe here, weakly calcified coccolithophorids, is largely unknown. This lack of information on their ecological significance stems in large part from our inability to enumerate them. Results based on epifluorescence microscopical filter counts (EFM) do not provide information on the specific contributions by unmineralised forms (e.g. *Chrysochromulina* spp. and *Phaeocystis* spp.) and coccolithophorids, respectively. However, by using other techniques (inverted microscopy of sedimented samples) the abundance of the latter have been found to be typically within the range 10^3 – 10^4 cells l^{-1}, whereas the entire haptophyte community typically exceeds 10^6 cells l^{-1} (Thomsen *et al.*, 1994). The weakly mineralised forms treated here are generally too inconspicuous to be enumerated from settled samples. The apochlorotic nature of the cells furthermore ensures that they are likely to be incorporated as heterotrophic flagellates in the EFM count statistics, and only recognizable as members of the Prymnesiophyceae if both flagella and haptonema are well preserved. However, the examination of whole mounts of cells prepared for light microscopy has for the Antarctic indicated likely cell abundances of $1\text{-}5 \times 10^3$ cells l^{-1} (Marchant & Thomsen, 1994). Realizing that these organisms tend to be most abundant in polar regions, their contribution to the unicellular biomass in samples from lower latitudes is probably low. The motivation and justification for further study of these particular types of coccolithophorids is not their contribution to carbon flow, but rather the unique position of this species complex within the Prymnesiophyceae. Their lack of chloroplasts indicate that organisms reminiscent of these are potential ancestors for the entire group (Cavalier-Smith, 1994) and their participation in complex life-histories involving holo — and heterococcolithophorid stages contributes significant new knowledge on the biology of the Prymnesiophyceae.

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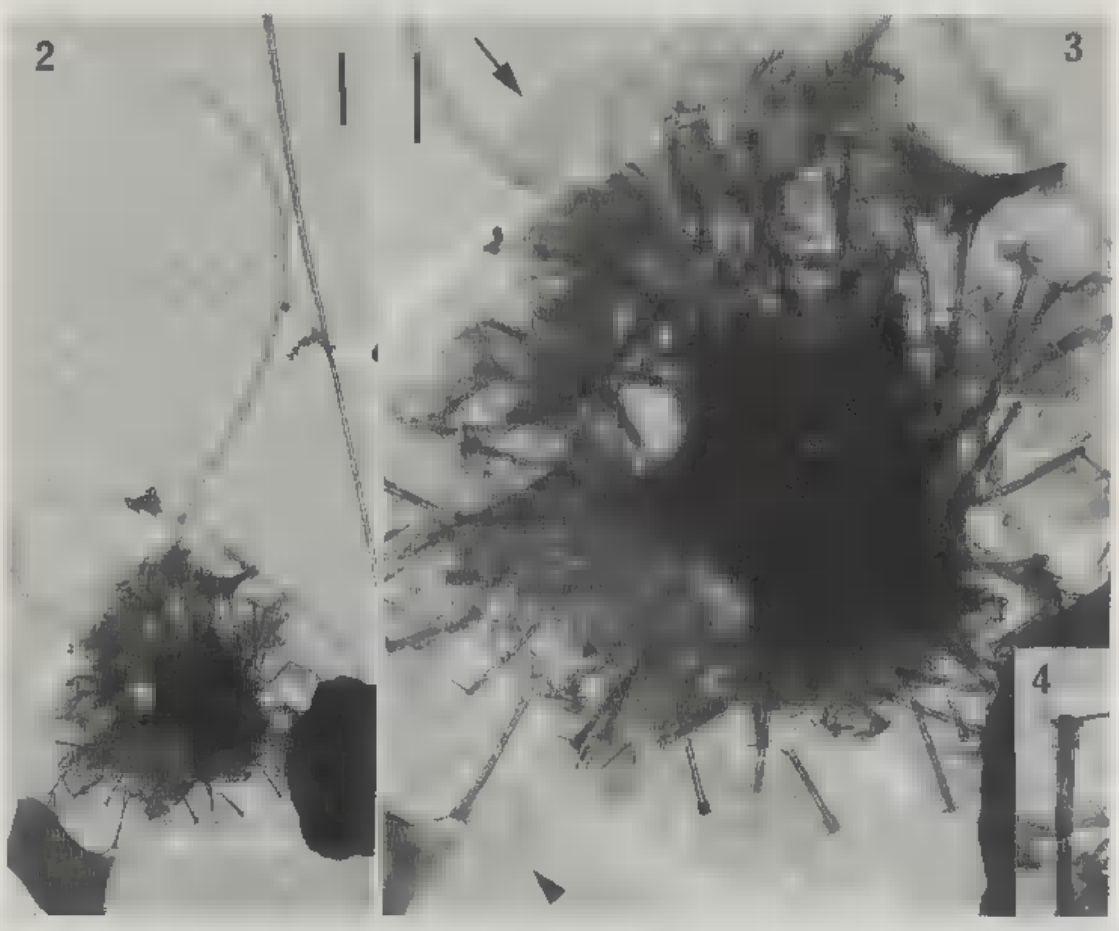
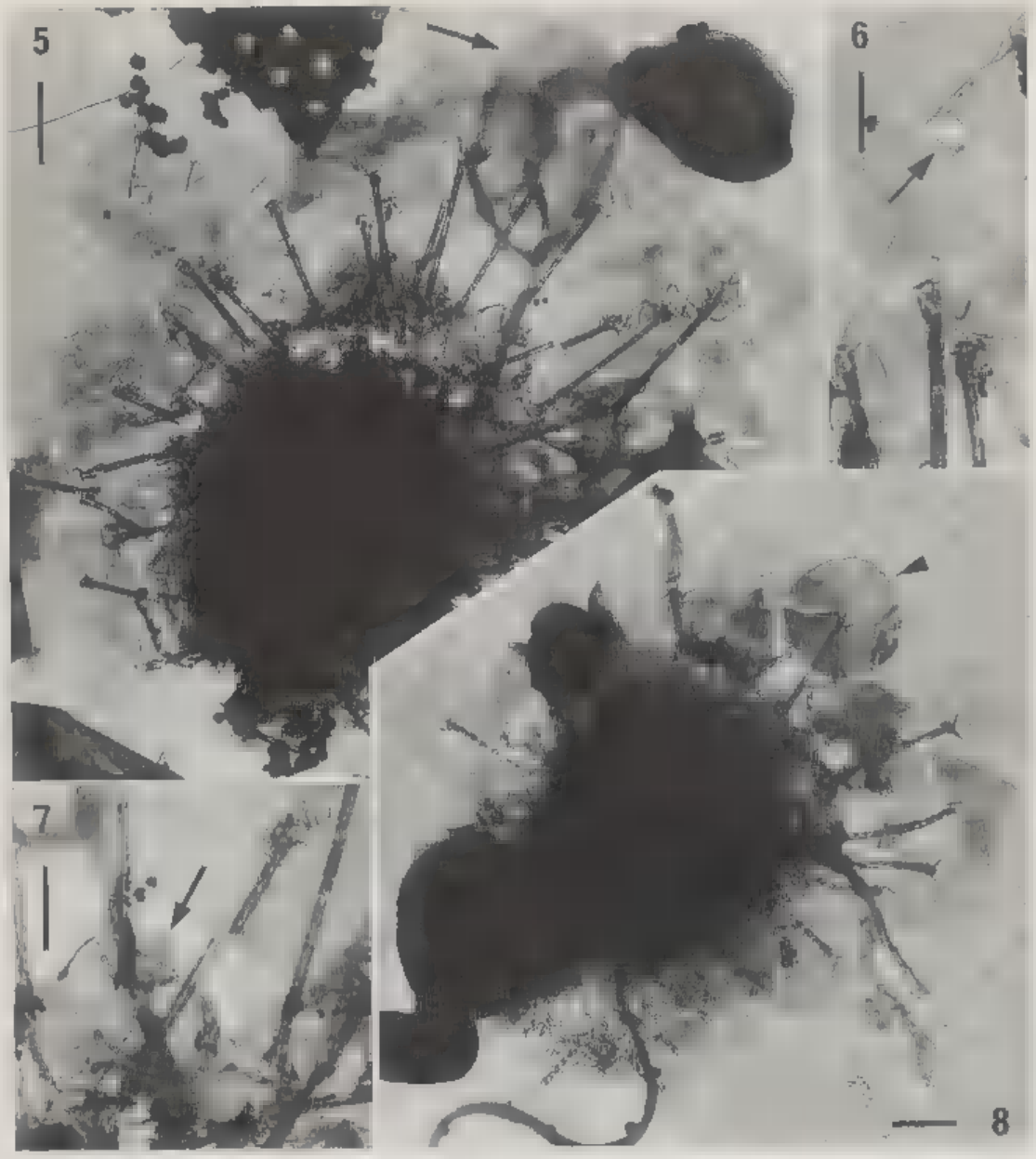
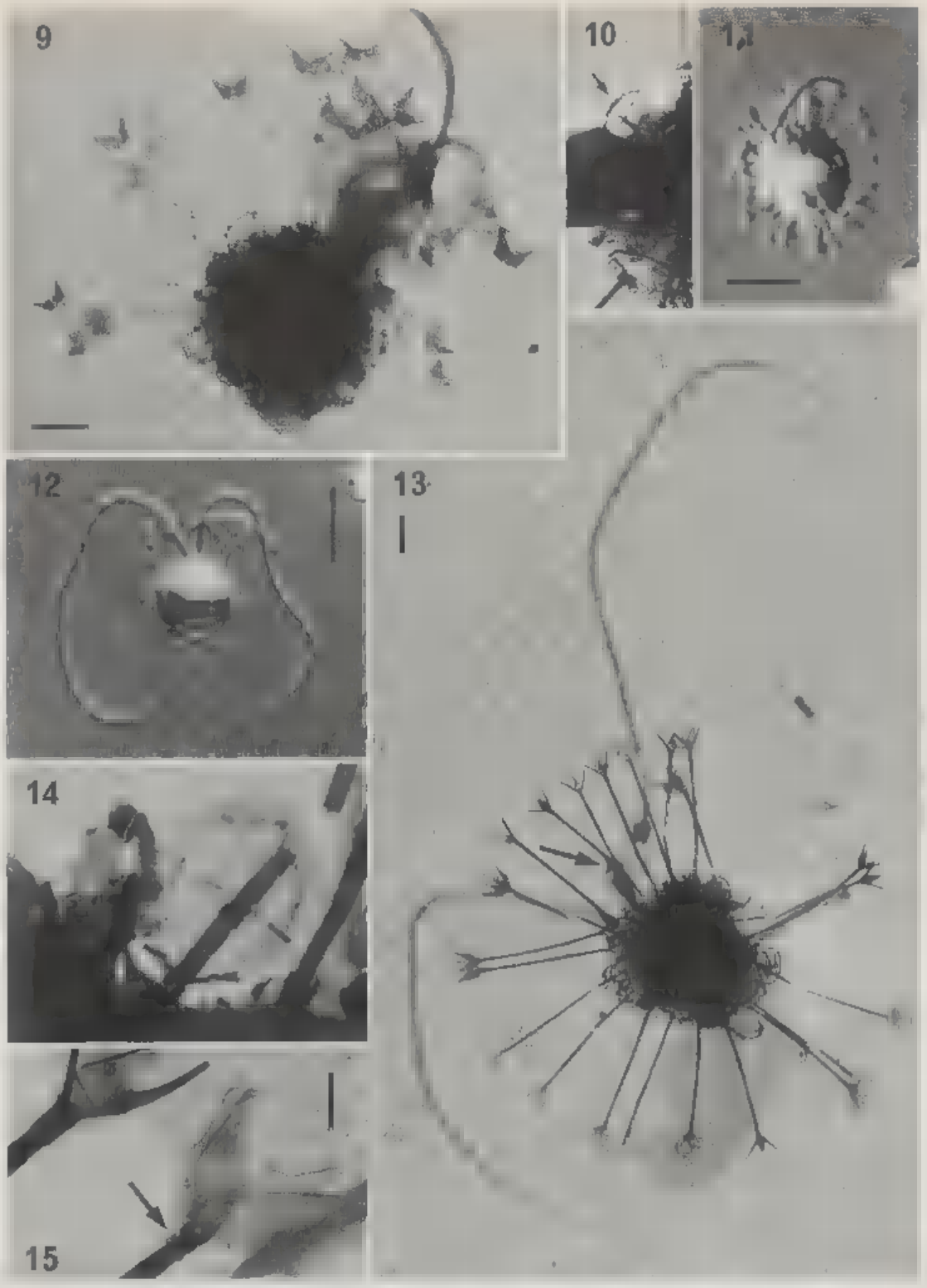


Figs 2-4. *Papposphaera lepida* from California; shadowcast whole mounts for TEM. Fig. 2. Cell with flagella. Fig. 3. High magnification of coccosphere. Notice the conspicuous size difference between apical (arrow) and antapical (arrowhead) cell end pappoliths. Fig. 4. Detail of process appendage (x 20,000). Scale bars: 1 μm (Fig. 3); 2 μm (Fig. 2).

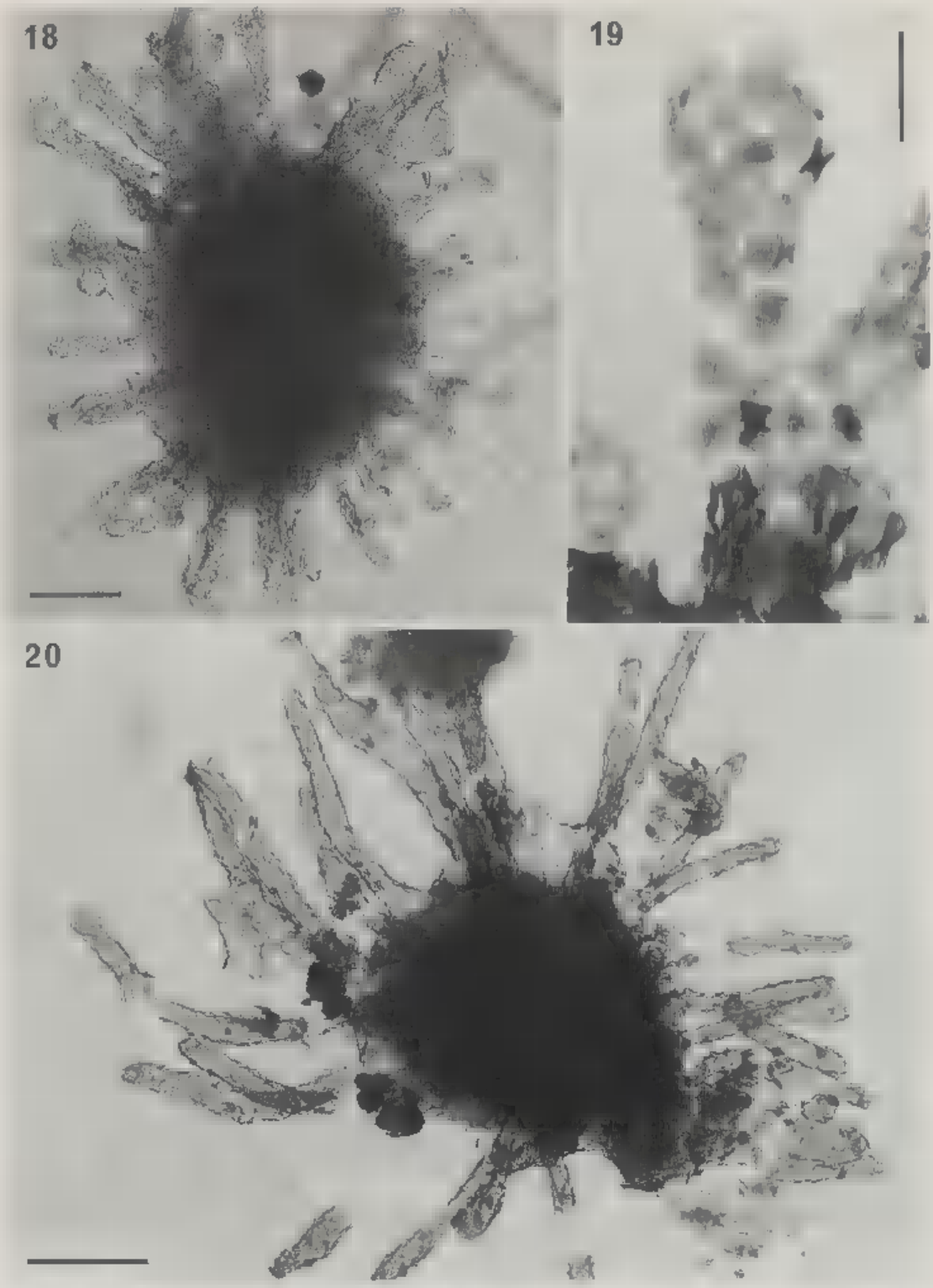


Figs 5-8. *Papposphaera lepida* from Mexico; shadowcast whole mounts for TEM. Fig. 5. Coccosphere with square process appendages only. Notice the coiling haptonema (arrow). Fig. 6. Details of process appendage. Notice wristlet and the displaced triangles forming a square central aperture (arrow). Fig. 7. Detail of pappolith base-plate calcification (arrow). Fig. 8. Coccosphere comprising a single discoid process appendage (arrowhead). Notice the two flagella and the conspicuous size difference between apical and antapical cell end pappoliths. Scale bars: 0.5 μm (Figs 6-7); 1 μm (Figs 5, 8).

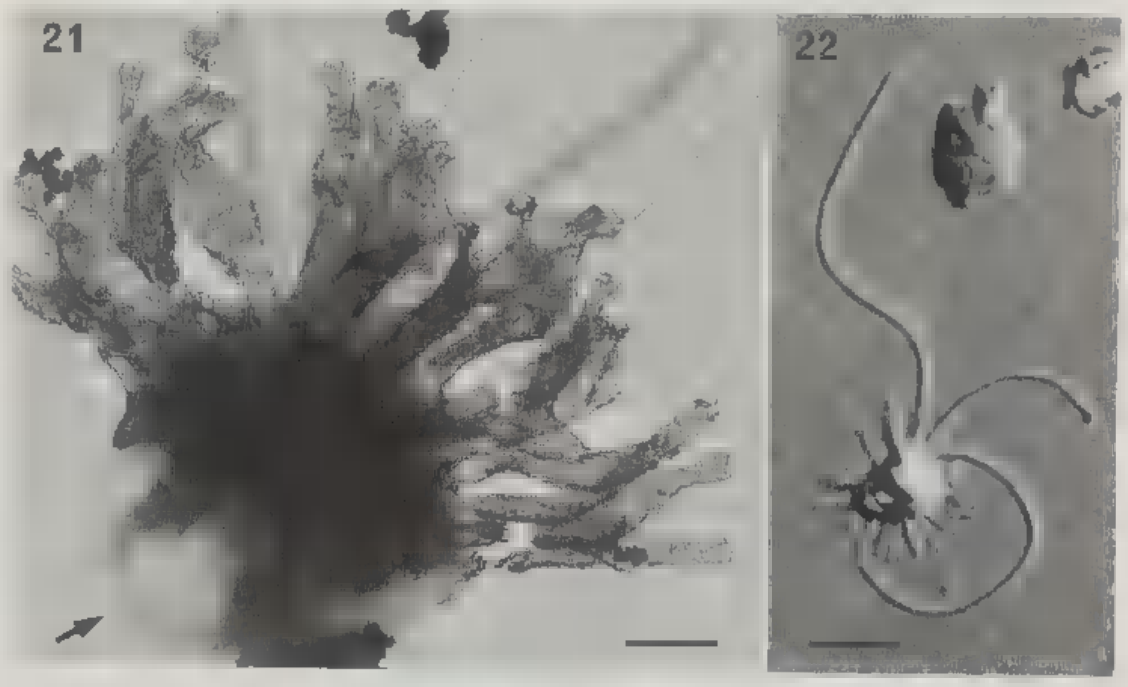


Figs 9-15. *Papposphaera bourrellii* sp. nov. from California (Figs 9-12), and San Juan Islands (Figs 13-15). Shadowcast whole mounts for TEM (Figs 9-10, 13-15); light micrographs (Figs 11-12). Fig. 9. Weakly calcified cell with intact flagellum and haptonema and conspicuous calyx-like process appendages. Fig. 10. Detail from normally calcified specimen; pappolith with cruciform base-plate calcification is indicated (arrow). For scale bar see Fig. 9. Figs 11-12. Light micrographs of Californian specimens (Fig. 11 is reproduced with permission from the Systematics Association). Fig. 13. Holotype (iconotype) specimen from McConnell Island with flagella and haptonema (arrow). Fig. 14. Detail of base-plate calcification (see text for further explanation). For scale bar see Fig. 15. Fig. 15. Detail of process appendages. The arrow points to the collar that is part of the interlocking devices between shaft and process appendage. Scale bars: 0.25 µm (Figs 14-15); 1 µm (Figs 9-10, 13); 5 µm (Figs 11-12).

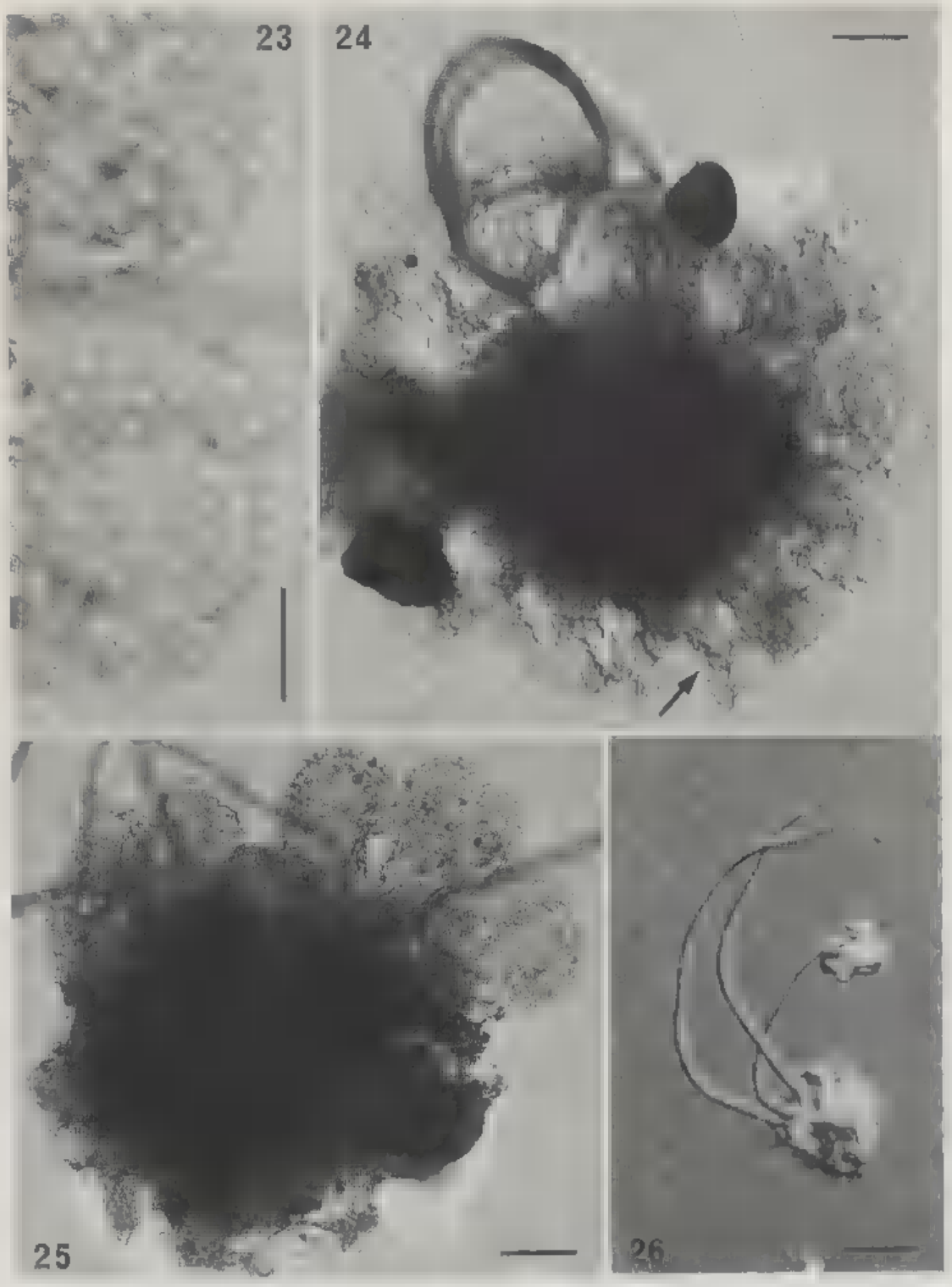
Fig. 16. *Papposphaera hourrellii* from Mexico. Light micrograph of specimen with flagella and haptonema; notice that the species identification is uncertain. Fig. 17. *Papposphaera* sp. 1 from Mexico. Shadowcast whole mount for TEM showing details of pappoliths. The arrow points to elements from the coccolith base plate rim. Scale bars: 0.25 µm (Fig. 17); 5 µm (Fig. 16).



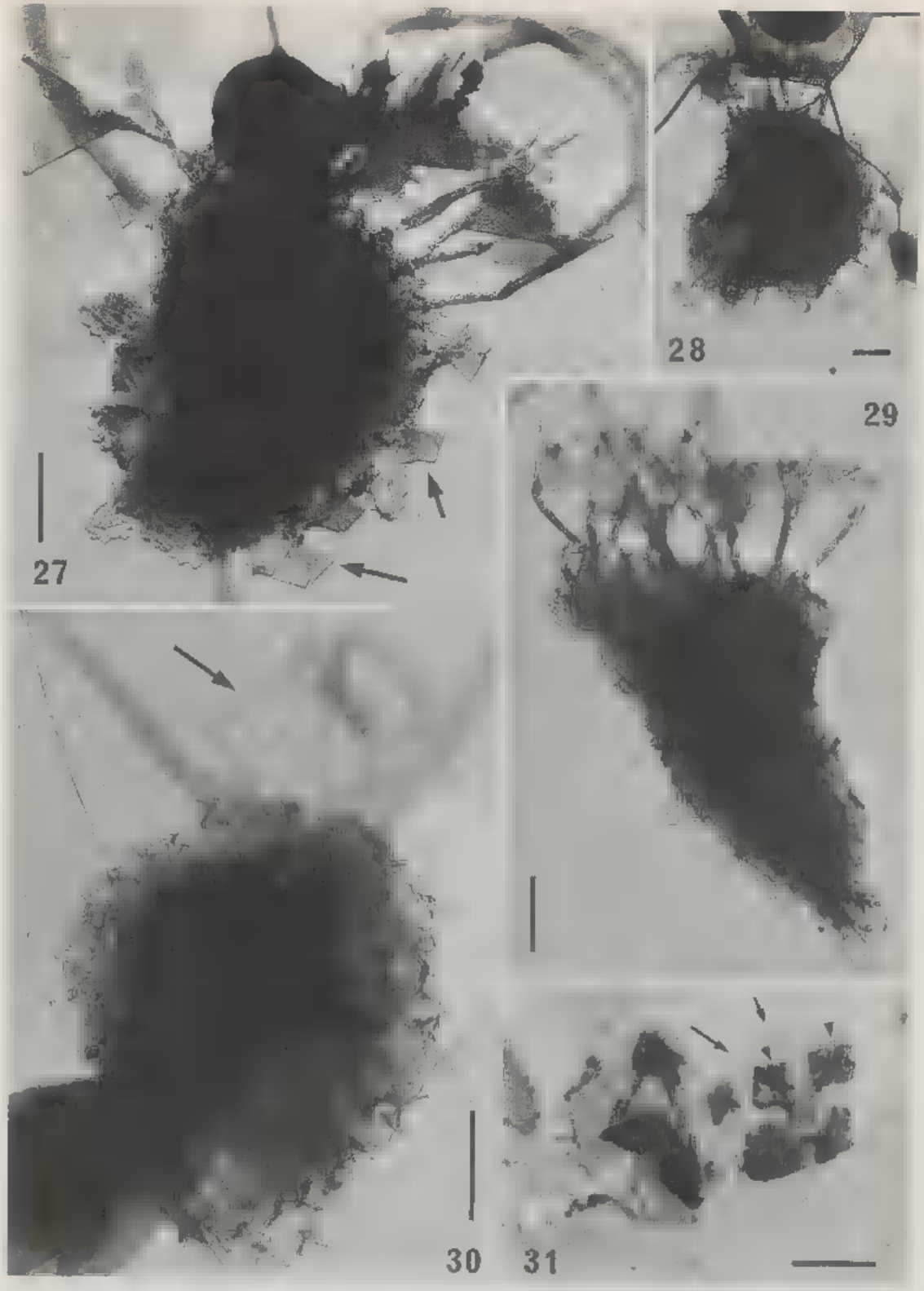
Figs 18-20. *Papposphaera* sp. 2 ("*Turrisphaera* phase") from Mexico; shadowcast whole mounts for TEM. Fig. 18. Cell with both flagella intact and ■ complete coverage of turriform coccoliths; notice the difference in overall coccolith length from one cell pole to the other. Fig. 19. Detail of coccolith showing hexagonal crystallites. Fig. 20. Cell with very long, thin and irregularly bending coccoliths. Scale bars: 0.25 µm (Fig. 19); 1 µm (Figs 18, 20).



Figs 21-22. *Papposphaera* sp. 2 ("*Turrisphaera* phase") from California; shadowcast whole mount for TEM (Fig. 21) and Nomarski light micrograph of dried cell (Fig. 22). Fig. 21. Broken coccosphere. The apical pole coccoliths are irregularly shaped and with weakly developed, distal, unilateral proliferations. An underlayer of minute, unmineralised scales is exposed at one end of the cell (arrow). Fig. 22. Complete cell with flagella and haptonema. Reproduced with permission from the Systematics Association. Scale bar: 1 μm (Fig. 21); 5 μm (Fig. 22).



Figs 23-26. *Papposphaera* sp. 3 ("*Turrisphaera* phase") from California; shadowcast whole mounts for TEM (Figs 23-25) and Nomarski light micrograph of dried cell (Fig. 26). Fig. 23. Detail of coccolith (from Fig. 25) showing the hexagonal crystallites. Fig. 24. Weakly calcified specimen. The arrow points to ■ symmetrically shaped coccolith from the antapical cell end. Fig. 25. Complete cell with flagella and haptonema intact; notice the unilateral, flaring proliferations of the apical cell end coccoliths. Fig. 26. Whole cell with flagella and haptonema. Scale bar: 0.5 µm (Fig. 23); 1 µm (Figs 24-25); 5 µm (Fig. 26).



Figs 27-29. *Pappomonas flabellifera* var. *flabellifera* from Mexico (Fig. 27) and California (Figs 28-29); shadowcast TEM whole mounts. Fig. 27. Complete cell; arrows point to antapical cell end pappoliths with reduced central processes. Fig. 28. Cell with numerous, reduced, antapical appendages. Fig. 29. Complete cell. Reproduced with permission from the Systematics Association. Scale bar: 1 μm (Figs 27-29).

Figs 30-31. *Polycrater galapagensis* from Mexico (Fig. 31) and California (Fig. 30); shadowcast whole mounts for TEM. Fig. 30. Cell complete with flagella and haptonema. Reproduced with permission from the Systematics Association. Fig. 31. Details of single coccoliths. Arrows point to petal-like elements forming a bowl. The subtending cruciform structure is identified with arrowheads. Scale bar: 0.25 μm (Fig. 31); 1 μm (Fig. 30).

A PROBLEM IN ALGAL ECOLOGY — CONTAMINATION OF HABITATS FROM ADJACENT COMMUNITIES

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ABSTRACT — Problems associated with the contamination of distinct diatom communities by live or dead frustules (valves) from adjacent communities are discussed. The importance of the recognition and discrete sampling of the numerous microhabitats in both freshwater and marine systems is stressed. Special comments are made concerning the complexity of the epilithic flora in rivers and the "metaphyton" associated particularly with the epiphyton.

RÉSUMÉ — Les problèmes associés à la contamination de communautés distinctes de diatomées par des frustules (valves) morts ou vivants ■ provenance de communautés adjacentes sont discutés. L'importance de la reconnaissance et de l'échantillonage séparé des nombreux microhabitats, aussi bien en eau douce que dans le milieu marin est souligné. Des commentaires particuliers sont faits sur la complexité de la flore épilithique des rivières et le "metaphyton" associé notamment avec l'épiphyton. (Traduit par la Rédaction)

KEY WORDS: Bacillariophyceae, contamination of communities, diatoms, epipelon, epiphyton, epipsammon, microalgae, plankton.

INTRODUCTION

Unlike the situation confronting higher plant ecologists, workers in micro-algal freshwater or marine ecology are faced with admixtures of live or dead cells from several adjacent communities within their chosen micro-habitat.

Sampling of microscopic organisms – unlike higher organisms — usually involves sampling ■ sector of the physical habitat, e.g. a litre of water, an area (volume) of sediment, an area of rock or plant surface. But movement in the surrounding water deposits live (and dead) organisms from numerous niches into the chosen niche. Experience of the range of habitats enables workers to detect these and make appropriate allowances. Nevertheless, the literature abounds in records of contaminants, e.g. of benthic taxa in plankton lists and *vice versa*.

The problem is even more acute where listing or counting of unicells involves species which have cell walls which are resistant to decay, e.g. desmids, diatoms, coccolithophorids, *Pediastrum*, *Scenedesmus*, *Trachelomonas*, Dinoflagellate and Chrysophyte cysts, etc.

Few papers discuss these problems and it is only by studying the data in papers and from working continuously with samples taken from precise habitats that the problems surface. Two features strike me as major problems. The first is that precisely chosen and described habitats (other than the plankton) are rarely adequately sampled and secondly, deriving from the crude sampling the exact habitat of many microalgae is unknown — it certainly rarely appears in modern floras where one might expect this information.

Contamination (defined by the Oxford English Dictionary as "to render impure by contact or mixture"), is not easily detected in aquatic systems without complete knowledge of all the associated floras, but at least the obvious impurities can be removed, e.g. by separating silt and sand from plant surfaces and stones. At the same time planktonic species can be separated. The sediment community (epipelon) can be sampled by utilising the phototactic movement of species out of the sediments thus isolating the live cells from the multitude of dead cells. Merely studying the sediment mixes all the algal communities of the water body.

Diatoms *do* occupy distinct habitats and *do* form discrete associations but these are rarely defined since few workers make assessment of live cells, and when the sampled material is cleaned to make microscope slides/SEM preparations, all the casual cells confuse the true association.

Some aspects will now be considered in greater detail, concentrating on the diatoms.

CONTAMINATION OF COMMUNITIES

Paper after paper concerned with diatoms in the environment* contain lists of species which are present in small numbers. Why are they there? This is not always simple to answer, so let us consider some of the approaches. Many records are made from samples boiled in acids, thus no account can be taken of dead cells/valves swept up in the sampling. I will come to other problems of sampling later. Ecological studies ought to be based on live material, all diatoms should be identifiable by light microscopy of live material (see Cox, 1996). It is possible, though no comprehensive floras based on internal cell morphology exist and thus every worker has to make their own flora. One of the tragedies of diatom studies is the almost total lack of consideration of live cells both in ecology and taxonomy: no other algal group suffers this massive hindrance. Theoretically and often practically all diatom communities contain dead cells of the actual community (in the epilithon it is quite common to encounter samples in which almost all the cells are dead) but also varying proportions of dead cells from other communities — the former confuse the instantaneous population data — the latter are just irrelevant. E.g. in a recent study (involving planktonic diatoms of a lake), 10 out of 47 species listed were truly planktonic — it is difficult to see how this happened if sampling was in mid-lake and with uncontaminated apparatus. One could envisage situations where flocs of diatom contaminated material occurred in the open water but these should be obvious and noted as such, not recorded as plankton. The benthos is however a much larger problem. All habitats may be bathed in planktonic material which cannot be avoided by any conceivable sampling technique — the

* (I hesitate to use the term diatom ecology – ecology is the most difficult aspect of biology, the last to be tackled only after all other aspects have been put in place, including the physics and chemistry of the environment – most so-called ecology is merely listing species).

only solution is the usually easy recognition and discarding of planktonic cells in the analysis, e.g. river epipelon is at certain times of the year overwhelmed by *Nitzschia acicularis* (Kütz.) W. Sm. deposited from the water column. But also each benthic habitat (see below) can receive cells from other benthic habitats and these must somehow be screened out. What often happens is that counts are made on acid cleaned material (it is difficult to do otherwise) and a percentage limit set to discard minor components. Strangely it seems true that contaminants, though always present, are never as abundant as one might expect from theoretical considerations! A fortunate situation. But a problem arises here since detailed sampling often reveals that minor components consistently occur in certain environments, e.g. *Navicula integra* (W. Sm.) Ralfs is never abundant in British river epipelon but at some stations low down the rivers it occurs in relatively small cell numbers and must reflect the conditions within these reaches. Only lengthy detailed sampling will reveal such critical aspects of diatom ecology. Many other rare species are, however, simply washed downstream — these can only be determined by sampling at close intervals — or preferably, devising suitable techniques for counting live cells. In general, in my experience, live cells are rarely transported into other communities in any quantity — they tend to die rapidly when removed from their intrinsic niche.

This problem is of course multiplied when mixed communities are sampled, since not only two (or more) discrete communities are recorded as one, but the contaminants of both are added in. What is a mixed community? Each worker must decide this himself, but see below.

Some habitats act as traps for casual species, e.g. mosses such as *Fontinalis* contain a mixture of species. The epipelon lives of course amidst a deposit of species from other habitats but to some extent the motile cells can be at least concentrated and removed from the sediment by various techniques, though it is difficult to make a total quantitative collection of the epipelon and at the same time eliminate contamination.

ON TAXONOMY/ECOLOGY

It is useful when deciding on the identity and systematic position (generic) of a taxon to ask about its ecology. At the most basic level, marine or freshwater — an apparently elementary question, quite so, but nevertheless a fundamental one. But what is the proper question? I believe it is "where is the centre of distribution of the taxon in question"? Is it in water of a salinity of 0.1 to 2.0‰ (*i.e.* you would drink it) or is it above this and more likely approaching 35‰ (in which case you would not drink it). Arguments about the region around 2.0‰ are relevant in special areas, e.g. the Baltic coasts and it is essential in these situations to make very careful studies of the tolerance of each and every species. Here it is worth bearing in mind the single most important sentence I can find in the one and only book I know of entitled *Ökologie der Diatomeen, i.e.* Cholnoky's 1968 publication. Here he states "ecology is the study of the physiology of tolerance"— though not here in relation to salinity tolerance but the much more subtle effects of nitrogen heterotrophy and other more controversial aspects. Equally, but much simpler than in the Baltic situations, is the salinity encountered in the very common, especially but not inclusively in the semi-arid/arid regions of the land masses where evaporation produces inland saline lakes. Here the indicator species are well known and well documented.

To return to the freshwater/marine situation, an attempt was made by Pat Sims and myself to indicate the extent to which genera are exclusive to (or better have their centre

of distribution in) freshwaters or marine habitats. Since this was published in 1981 it has become increasingly clear that our earlier statement that "the exceptions to the rule" are the result of inadequate taxonomy has proved true. For example. *Navicula* now contains only freshwater genera of the old section Lineolatae, *Nitzschia* has had several sections removed thus clarifying its freshwater centre of distribution (work still remains to be done).

Within the freshwater environments a trace of salinity, purely of marine origin (e.g. sea spray) will result in the occurrence of *Ctenophora pulchella* (Grun.) Williams & Round – further evidence for its individual status outside *Synedra* which cannot tolerate saline water. Alternatively "pollution" by industrial/agricultural input of salts also results in the occurrence of *Ctenophora* but more commonly of species such as *Caloneis amphisbaena* (Bory) Cleve, *Cyclotella meneghiniana* Kütz., making an increased contribution to the flora. Natural inland salt (not necessarily NaCl) accumulation (evaporation basins), salt springs, etc. will produce floras dominated by *Anomoeoneis sphaerophora* (Kütz) Pfitzer and *Craticula cuspidata* (Kütz) D.G. Mann. What is the effect on the diatom physiology — is it an osmotic effect or the mixture of salts or something more specific? Every species distribution contains clues to its environmental requirements.

Drainage from road salt stored for winter de-icing can leak into acid streams and promote the growth of *Ctenophora pulchella*, e.g. at a site on the River Wye which is normally dominated by the acid-loving *Eunotia exigua*. It is however most important to note that all these species are in no sense "marine" in distribution. Examples such as this indicate a positive requirement for a particular element(s) - - but for what exact purpose — experimental studies are desperately needed.

IMPORTANCE OF MICROHABITATS

The precise place in which diatoms live is important and should be recorded very specifically. The plankton is the only habitat where this is at its simplest. In general, in freshwater a net sample taken some distance offshore will be adequate and uncontaminated. However, there are complications which should be considered. Diatoms can collect at various depths during lake stratification and in oceanic regions populations can lie on the thermocline and in the upper surface populations can be affected by insolation at the surface and/or sinking from the surface. Layering is not confined to buoyant/motile species — diurnal movements can be significant. In some situations, non-planktonic diatoms associated with zooplankton either directly attached (e.g. *Protoraphis* and *Pseudohimantidium*) or associated with mucilage aggregates (involving living organisms or as organic flocs) can support communities which are as yet relatively uninvestigated. There is still much work to do even on the apparently simple plankton.

The benthos is infinitely more complex and affords a wealth of fascinating problems. It is all too easy to assume that diatom species colonise microhabitats indiscriminately. Just because a few species "appear" to be widespread is misleading. Because the benthic habitats tend to be adjacent and form mosaic patterns of colonisable sites, great care has to be taken to sample individual microhabitats. Most diatom floras have general comments and these are often misleading. Consulting slides made from acid cleaned material is usually equally confusing though slides made from carefully sampled communities can provide reliable data on community structure. Since sampling microhabitats in nature is often difficult, let me start with one of the easiest and as is so often helpful, proceed from the simple to the complex. The epipsammon, especially the marine, is easy to sample; a tube of sand

from a beach is sufficient. This will of course be contaminated by deposited plankton but this can be washed out by shaking and settling in water (tap water will do). The removal of all organic particles is necessary since they often contain old or even viable diatoms. The sand can be treated with HCl to dissolve shell fragments and with other acids, etc. to remove the numerous epipsammic diatoms (e.g. *Anorthoneis* spp., very small *Amphora*, *Navicula*, *Cocconeis* spp.) attached to the sand grains. However, do not throw away the first washings — they often contain a motile flora (*Hantzschia* is very common) — presumably an endopelic community as yet unworked ecologically. As with the plankton this is a simplified account. On some beaches there is a diatom flora in the surface layer of sand grains and another below the surface (endopsammic — Round, 1979). Diurnal movement of the endopelic component is commonplace (Round & Palmer, 1967). A warning! Some beaches seem to be quite barren but this is often misleading. And as one moves into estuaries the epipelic flora of muddy sites is mixed with the epipsammic. The epipelic can be concentrated by placing coverglasses on the surface of the sand in a Petri dish (as can the *Hantzschia* mentioned above). To avoid contamination picked up in organic material attaching to the coverglass, these can be lain on a layer of lens tissue, or a second layer of lens tissue placed on top of a first layer can be used, but such techniques require careful study in each environment since no technique is completely reliable in harvesting every species of the motile epipelon.

An interesting experience I had recently. Sampling a river draining carboniferous limestone — the stones were encrusted with several millimetres of blue-green algal mucilage/carbonate particles. The outside was coated with *Cymbella minuta* Hilse/*Nitzschia* spp, internally a layer of blue-green algae was rich in dead, empty valves of *Cymbella*/*Nitzschia*, etc. but was also rich in live *Achnanthes* spp. and a lower layer was devoid of diatoms as was the final layer adjacent to the stone surface. This was merely a casual observation and requires much more accurate detailed sampling.

The plankton, epipsammon and epipelon have been dealt with above and all that remains is the epiphyton and epilithon. Both these are complex and collections from the field are rarely pure. The epiphyton can be observed directly and live cells counted. Higher plants, mosses and liverworts can be contaminated by diatoms disturbed by currents, etc.; flocculent material can be deposited — all this can be washed off, collected and assessed and the clean plant material treated to remove the attached epiphytic community (some loosely attached epiphytes may wash off with contaminants and care must be taken to remove the firmly attached adnate species such as *Cocconeis* – the raphid valves are often glued tightly to leaf surfaces and methods must be devised to cope with counting — there are few short cuts!! A more critical problem arises where the plant surfaces are colonised by epiphytic filamentous algae, e.g. *Oedogonium*, which may itself be colonised by *Cocconeis pediculus* Ehr. when the host Angiosperm is colonised by *C. placentula* Ehr. Large seaweeds are a much greater problem — since complex communities of filamentous and thalloid species often grow on the larger host plant — they simply have to be dissected out under a binocular microscope and some loss/contamination is difficult to avoid — careful recording of live diatoms on the various epiphytes can at least give initial information on distribution — there is absolutely no doubt that specific floras are involved (e.g. Harper & Garbary, 1994 discovered *Podocystis adriatica* (Kütz.) Ralfs on the red alga *Heterosiphonia crispella* (C. Ag.) Wynne, 1995) and also that some marine algae are devoid of diatom epiphytes. A simple freshwater example of the care needed is the fact that the undersurface of *Lemna* leaves support *Achnanthes hungarica* (Grun.) in Cl. & Grun. but the roots do not. Why is *A. hungarica* reported as confined to *Lemna*? In fact, it is not — only recently did I think to sample *Azolla* fronds and it exists there too — but why only on the small floating plants?

The epilithon is much more complex and most papers usually purporting to relate the diatom epilithon to levels of pollution, etc. are hopelessly confused. Firstly there is a range of stones in rivers from clean to complexly colonised, *i.e.* from "primary" sites to "climax" communities. The stones can be devoid of deposited sediment or coated with variable layers — on and in this a proportion of the river epipelon can grow, *i.e.* an epipelon can simply exist on a stone surface above the epilithic community. However, a word of warning — in my experience, only a proportion of the epipelic species grow in profusion on the silted stone surfaces, so here there is some selective mechanism operating. The epilithon should be thought of in terms of a forest often embedded in mucilage. The "trees" are stalked species of *Cymbella*/*Gomphonema*, the "shrubs" of stalked *Achnanthidium*, the "herbs" of adnate species, *Epithemia*/*Cocconeis*, etc. How do we separate these? First wash off the epipelon, gently brush off the "trees", layer by layer (!), finally scrape off the adnate species with a razor blade. Often the layers are extremely thick and in alkaline situations complicated by calcareous deposits and other microscopic algae. Worse is to come! Many epilithic samples are further confused -- the epilithon can and frequently does include attached macroalgae, e.g. *Cladophora*, *Oedogonium*, *Lemanea*, etc. — all with their own specific epiphytes. These can be removed but often the basal branching system and short upright branches remain and these are the oldest regions and often thickly coated with epiphytic (*not* epilithic) diatoms — a major species being *Rhoicosphenia curvata* (Kütz.) Grun. and I am not sure whether this taxon ever grows on stone surfaces. Worse still is to come. The most difficult and therefore virtually unknown diatom flora is that of solid rock surfaces in waters of all types — if they are exposed, then at least they can be scraped with a sharp blade — collecting the scraping is difficult. What is the answer? Certainly not artificial substrata unless one is interested in fouling of unnatural surfaces. The aim of the ecologist is to describe and determine the functioning of *natural* communities. There is no doubt that they are more complex than I have described in this short survey and they are infinitely more complex than the artificial assemblage on glass slides — diatom ecology is in its infancy — in fact, as a diatom ecologist myself, I would say it does not yet exist apart from a few studies. Is it so complex that synecology should give way to autecology? — certainly the latter approach is easier and can be achieved without too much taxonomic complexity.

But the future is not all gloom, not at all. The field is completely open — 99% of the work remains to be done. Research students should ignore the system which makes them perform literature surveys for months before commencing work — a sure way to get onto the wrong track or give up altogether. Better — imagine you are a diatom, and look at the problem through the eyes of a diatom and devise your sampling, etc. to answer the question you ask of the community.

THE METAPHYTON

In a classic but sadly neglected study (Behre, 1956), the term "metaphyton" was coined for the loose flocculent community living amongst algae, especially prominent in the mucilaginous flocculent material around the stems of *Equisetum*, *Phragmites*, etc. in ponds and lakes. This is an actively growing, specific flora forming a self-standing algal association, though it may harbour some contaminants — a feature which requires further study. There is a possibility that a metaphyton occurs also in the epipelon and epiphyton of

rivers, e.g. extensive sampling of these communities over several years has revealed a "contaminant" flora of motile (*i.e.* usually epipelic) diatoms in both communities. But it is a striking feature that the majority of epipelic species (e.g. *Navicula capitata* Ehr., *Sellaphora pupula* (Kütz.) Meresch., *Placoneis elginensis* (Greg.) Cox, *Surirella brebisonnii* Kram. & Lange-Bertalot, *Stauroneis smithii* Grun., *Neidium dubium* (Ehr.) Cl., *Caloneis silicula* (Ehr.) Cl., *Amphora ovalis* Kütz., *Nitzschia sigmoidea* (Nitzsch.) W. Sm. to name just obvious easily identifiable forms), are never found in the epiphyton but *Navicula tripunctata* (O.F. Müll.) Bory and *N. lanceolata* (Ag.) Ehr. are commonly found. At first I believed these latter two taxa were contaminants derived from the epipelon but further analysis showed that *N. tripunctata* is only a minor component of the epipelon (though always recorded at the majority of sites), and therefore I am forced to conclude that its favoured habitat is the epiphyton or, since it is motile, perhaps forming a metaphytonic component together with a few other motile *Navicula* species. Since all the dominants in the epiphyton are stalked or attached by mucilage pads and therefore non-motile, it has always been my assumption that any motile pennate diatoms found in epiphytic and even in epilithic communities were contaminants, but perhaps this view has to be revised. *Navicula lanceolata* remains a problem, however, since it is obviously a dominant, motile diatom in the epipelon and it is well represented in numerous samples of epiphyton and epilithon — though washing of plants and stones does reduce its presence. There is a need to check unsilted epiphyton and epilithon where it is less likely to be present. At the moment my inclination is to favour the epipelon as its natural habitat. This data does not mean that care must not be relaxed when studying the attached communities and even greater attention must be paid to distinguishing between contaminants and possible metaphyton.

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RAPHE VESTIGES IN "*ASTERIONELLA*" SPECIES FROM MADAGASCAR: EVIDENCE FOR A POLYPHYLETIC ORIGIN OF THE ARAPHID DIATOMS?

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Dedicated to the memory of Pierre Bourrelly

ABSTRACT — Endemic *Asterionella* taxa from Lake Tsimbazaza, Madagascar, were studied from type material with light and scanning electron microscopy and shown to possess short raphe branches; rimoportulae may be present or absent. These taxa appear to be closely related to *Actinella* species also endemic to Lake Tsimbazaza, by virtue of symmetry and raphe features. Together, these "*Asterionella*" and *Actinella* taxa from Madagascar appear to be part of an early lineage within *Actinella*. Phylogenetic analysis suggests there was a progressive reduction in raphe length and structure in this lineage, as well as the development of stellate colonies. The latter feature may have been in response to the planktonic habit these forms now exhibit. Entire loss of the raphe system in other raphe-bearing groups (documented within the rhaphidioids as well as other raphe-bearing lineages) would yield araphid diatoms. Primitive and secondarily-derived origins of the araphid diatoms would make this group a morphological grade rather than a phylogenetic clade. If phylogeny is to be represented in taxonomy, we suggest the araphid diatoms *sensu lato* do not merit taxonomic recognition as a group.

RÉSUMÉ — Des taxons d' *Asterionella* endémiques du lac Tsimbazaza, à Madagascar, ont été étudiés à partir du matériel type, en microscopie optique et à balayage. Il est apparu que leur frustule possédait de courtes ramifications du raphe, des *rimoportulae* pouvant être présentes ou absentes. Ces taxons sont apparus étroitement apparentés aux espèces d'*Actinella* endémiques du lac Tsimbazaza, en raison de la symétrie et des caractéristiques du raphe. L'ensemble de ces "*Asterionella*" et de ces *Actinella* de Madagascar apparaît comme une partie d'une lignée ancienne au sein du genre *Actinella*. L'analyse phylogénétique suggère qu'une réduction progressive de la longueur et de la structure du raphe, ainsi que le développement de colonies étoilées se sont produits dans cette lignée. Cette dernière caractéristique pourrait constituer une réponse à l'habitat planctonique que ces formes exhibent désormais. La perte totale du système de raphe chez les autres groupes présentant un raphe (étudié au sein des raphidioïdées aussi bien que chez les autres lignées possédant un raphe) aurait conduit aux diatomées araphides. Des origines multiples, primitives et secondairement dérivées, des diatomées araphides auraient fait de ce groupe un gradient morphologique plutôt qu'un clade phylogénétique.

Si la systématique se doit d'être phylogénétique, nous suggérons que les diatomées araphides *sensu lato* ■ soient pas reconnues comme un groupe taxinomique. (Traduit par la Rédaction)

KEY WORDS: *Actinella*, araphids, *Asterionella*, diatoms, Madagascar, phylogeny, polyphyly, rhaphidioids, taxonomy, ultrastructure.

INTRODUCTION

Manguin (*in* Bourrelly & Manguin 1949) described 6 new *Asterionella* taxa endemic to Lake Tsimbazaza, Madagascar. These include:

Asterionella candelabrum Manguin *in* Bourrelly & Manguin
A. candelabrum f. *baculata* Manguin *in* Bourrelly & Manguin
A. madagascariensis Manguin *in* Bourrelly & Manguin
A. madagascariensis var. *madagascariensis* f. *osseiformis* Manguin *in* Bourrelly & Manguin
A. madagascariensis var. *minor* Manguin *in* Bourrelly & Manguin
A. madagascariensis var. *tibiaeformis* Manguin *in* Bourrelly & Manguin

Körner (1970), in his monograph of the genus *Asterionella*, considered Manguin's species of dubious taxonomic placement within the genus. Although unable to examine original material (or, actually, any material) of these taxa, Körner suggested Manguin's taxa were synonyms of other *Asterionella* species (e.g. *A. candelabrum* = *A. ralfsii* var. *americana* Körner) or of other genera (e.g. *A. madagascariensis* var. *tibiaeformis* = *Eunotia zasuminensis* (Cabejszekowna) Körner). Both species were reported from nearby Mauritius by Coste and Ricard (1984).

Manguin's original material from Lake Tsimbazaza has been located in the Laboratoire de Cryptogamie at the Muséum National d'Histoire Naturelle, Paris (PC), and has served as the basis of a previous paper on *Actinella* Lewis species endemic to this lake (Kociolek *et al.*, 1997). In this paper we provide light and scanning electron microscope observations on valve variation and ultrastructure of Manguin's *Asterionella* species from Madagascar.

MATERIALS AND METHODS

For light microscope (LM) observations of valve components, collections were cleaned in 30% H_2O_2 according to the procedure of van der Werff (1955). Cleaned material was washed with distilled water and settled repeatedly, then air dried onto coverglasses. Coverglasses bearing the dried material were mounted onto glass slides with Naphrax. Material used in all observations of taxa from Madagascar is from Lake Tsimbazaza; PC, "leg. Boiteau 2:3 and 2:4S3"; this is the original material used in the treatment by Manguin (*in* Bourrelly & Manguin, 1949). SEM observations were made on cleaned material air-dried onto coverglasses. The coverglasses were mounted onto aluminum stubs and coated with approximately 20 nm of gold-palladium. Coated stubs were viewed on a Hitachi S520 SEM. Striae measurements, as well as terminology of ultrastructural features are in accordance with standards proposed by Anonymous (1975) and Ross *et al.* (1979).

RESULTS

Asterionella candelabrum **Manguin *in* Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 58**
Figs 1-14

Light Microscopy. Valves are asymmetrical about the transapical axis, and also slightly asymmetrical about the longitudinal axis (Figs 3-6). The headpole is expanded and the apex has a slight notch. The footpole is narrow and rounded. The middle portion of the valve is expanded slightly. Striae extend across the valve; they are not interrrupted by a central sternum. Length, 26-43 µm, breadth 2 µm. Striae 18-26/10 µm.

SEM. External views show some valves may be slightly twisted about the apical axis (Fig. 9). Small spines are present on the margin but more prominent at the footpole. Round puncta extend across the face of the valve and onto the mantle. At the headpole (Fig. 10) and footpole (Fig. 11) a small slit-like opening can be found on the valve mantle of the ventral margin. The opening may extend onto the valve face (Fig. 12). Internally, at the both headpole (Fig. 13) and footpole (Fig. 14), small slit-like openings are found on one side of the valve at or near the face:mantle interface. The slit-like openings lack any additional structure.

Comment. The feature used by Manguin to segregate the taxon *A. candelabrum* f. *baculata*, namely a lack of swelling in the median part of the valve, could not be recognized. Thus we consider this taxon as part of the nominate form.

A. madagascariensis **Manguin *in* Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 60**
Figs 15, 16; 21-26

Light Microscopy. Valves are straight to almost C-shaped, bent to almost 90 degrees about the apical axis; asymmetrical about the transapical axis and, to a slight degree, about the apical axis. Headpole swollen, rounded; footpole rounded. Striae appear to extend across the valve face. Length, 50-70 µm, breadth 3 µm. Striae 18-20/10 µm.

SEM. Internal views show striae composed of round puncta that extend across the valve face (Figs 23-25). At the poles striae are arranged in a radiate fashion (Figs 22, 23, 26). A small rimoportula is positioned on the mantle at one end of the valve, and a small slit-like opening may be positioned on the valve face (Fig. 26). The slit-like opening differs from the rimoportula by lacking siliceous swellings on both sides along its longitudinal axis.

Comment. Specimens grade from straight to slightly bent to strongly arched. Valve shape was used Manguin to segregate *A. madagascariensis* var. *madagascariensis* f. *osseiformis* from the nominate. Given the continuum of variation expressed in valve shape, we consider the two taxa as synonyms.

***A. madagascariensis* var. *minor* Manguin *in* Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 62**
Figs 17-20; 27-30

Light Microscopy. Valves narrow, slightly asymmetrical about the transapical axis, distinctly asymmetrical about the apical axis, headpole bluntly rounded, footpole narrowly rounded. Terminal nodules are often distinct. Striae extend across valve face. Length 21-25 µm, breadth 1.5-2.0 µm, striae 20-24/10 µm.

SEM. Valves are shown to possess small, irregularly-placed spines along the periphery of the valve; spines are more concentrated at the apices than along the rest of the valve (Figs 27, 28). Round puncta comprise the striae, which extend across the valve face (Figs 27-29). Striae also extend onto the valve mantle. Small raphe branches are visible along the ventral margin (Fig. 27), restricted to the mantle except the extremity of the distal raphe end (Fig. 28). Internally the raphe terminates as helictoglossae, and a single, well-developed rimoportula is found on the mantle at one end only (Figs 29, 30).

***A. madagascariensis* var. *tibiaeformis* Manguin *in* Bourrelly ■ Manguin 1949, p. 166, pl. 5, fig. 63, pl. 6, fig. 64**

Figs 31-41

Light Microscopy. Valves only slightly asymmetrical about the transapical axis, distinctly asymmetrical about the apical axis. Ends bluntly rounded with a slight swelling at the middle part of the valve. Striae extend across the entire valve face. Length, 20-28 µm, breadth 1.5-2.5 µm. Striae 16-22/10 µm.

SEM. Valves are bent in the apical plane (Fig. 35). Heterovalvy is demonstrated with regard to the development of the raphe branch. On some valves the raphe may be distinct and extend from the mantle onto the face, in other valves the raphe may be small, irregularly arranged and restricted to the mantle (Figs 37, 38). Girdle bands are numerous and are of the open type. Some are continuous around the headpole and others around the footpole. Bands bear 1-3 rows of poroids; the poroids are of the same size and shape as puncta (Fig. 38). The valve face is bordered by small conical spines (Figs 36, 37). Internally, striae appear interrupted near the ventral margin, but continue onto the mantle. The raphe terminates as helictoglossae and at the footpole there is a single rimoportula (Figs 40, 41).

DISCUSSION

Considering the suite of features observed in the *Asterionella* taxa described by Manguin, including: a) presence of a raphe system, b) raphe system short, restricted to the mantle or just arching onto the valve face at the distal raphe ends and c) asymmetry about both the apical and transapical axes, it seems appropriate to assign these taxa to the genus *Actinella*. We therefore propose the following new combinations:

Actinella candelabrum **(Manguin *in* Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**
Basionym: *Asterionella candelabrum* Manguin *in* Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 58. Contribution a l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.
Synonym: *A. candelabrum* f. *baculata* Manguin *in* Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 59.

Actinella madagascariensis **(Manguin *in* Bourrelly & Manguin) Kociolek & Rhode, comb. [redacted]**
Basionym: *Asterionella madagascariensis* Manguin *in* Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 60. Contribution a l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.
Synonym: *Asterionella madagascariensis* var. *osseiformis* Manguin *in* Bourrelly & Manguin 1949, p. 165, pl. 5, fig. 61.

Actinella bourrellyi **(Manguin *in* Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**
Basionym: *Asterionella madagascariensis* var. *minor* Manguin *in* Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 62. Contribution a l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.

Actinella reviersii **(Manguin *in* Bourrelly & Manguin) Kociolek & Rhode, comb. nov.**
Basionym: *Asterionella madagascariensis* var. *tibiaeformis* Manguin *in* Bourrelly & Manguin 1949, p. 166, pl. 5, fig. 63, pl. 6, fig. 64. Contribution a l'étude de la flore algale d'eau douce de Madagascar: Le Lac Tsimbazaza. *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 161-190 + pls 1-7.

Within *Actinella*, these species appear closely related to other endemic *Actinella* from the same lake (Fig. 42). Kociolek *et al.* (in press) showed that the Lake Tsimbazaza taxa are primitive within *Actinella*, all lacking an apical point at the headpole. Amongst these primitive members of *Actinella*, it would appear that raphe reduction, including reduction in size and reduction/loss of helictoglossa is a derived feature. Many examples exist elsewhere of the secondary reduction/loss of raphe systems, including that for monoraphid diatoms (e.g. Andrews, 1981), *Navicula* Bory (Hustedt, 1962; Lange-Bertalot & Le Cohu, 1985) and rhaphidioid taxa such as *Peronia* Brébisson & Arnott *ex* Kitton (Gemeinhardt, 1926) and *Eunotia* Ehrenberg (Hustedt, 1952). Reduction of the raphe system in *Actinella* species might be related to its evolution from a benthic to a planktonic life form strategy. Species with more typical rhaphidioid raphe systems exhibit zig-zag colonies, similar to other *Actinella* species (e.g. *A. punctata* Lewis) while those with reduced raphe systems have stellate colonies (Manguin *in* Bourrelly & Manguin, 1949). A proposed set of relationships within this set of *Actinella* species is presented in Fig. 43.

If we can envision a total loss of the raphe system in *Actinella*, as suggested to have happened within *Eunotia* several times (Hustedt, 1952) and in *Navicula* (Hustedt, 1962), this would be additional evidence to suggest a polyphyletic origin of the araphid condition. Such a view opposes the traditional systematic placement of the araphids as a primitive group from which the ur-raphid diatom was to emerge. Proponents of such a view included Berg (1948) and Kolbe (1956), and it is inherent in the classification scheme

of Round *et al.* (1990) where the araphids are recognized as a separate class (similar to the scheme proposed by H.L. Smith, 1872). Primitive and secondarily-derived origins of the araphid diatoms would make this group a morphological grade rather than a phylogenetic clade. A polyphyletic origin of the araphids has received recent support from molecular data, where Sorhannus *et al.* (1995) showed that some araphids may be primitively so, while others, including "*Asterionella*" may be more derived.

Our classification systems should reflect the evolutionary relationships of the organisms they represent. The current system of recognizing a separate class for the araphid diatoms, given the evidence across many groups for their polyphyletic origin, would appear to be in conflict with this goal for classifications. We advocate recognition of only monophyletic groups in classification schemes, and therefore suggest no formal taxonomic designation for the araphids *sensu lato*.

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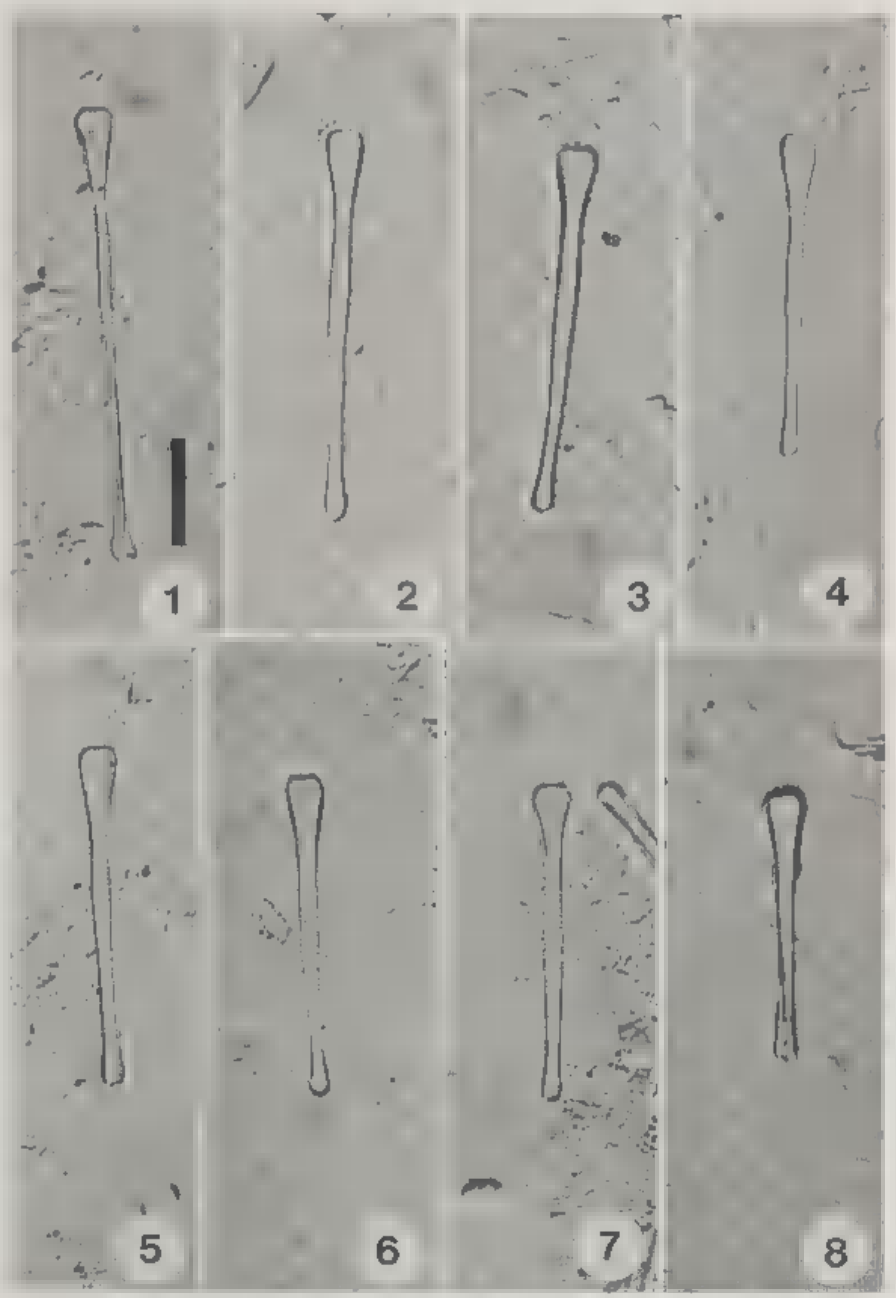
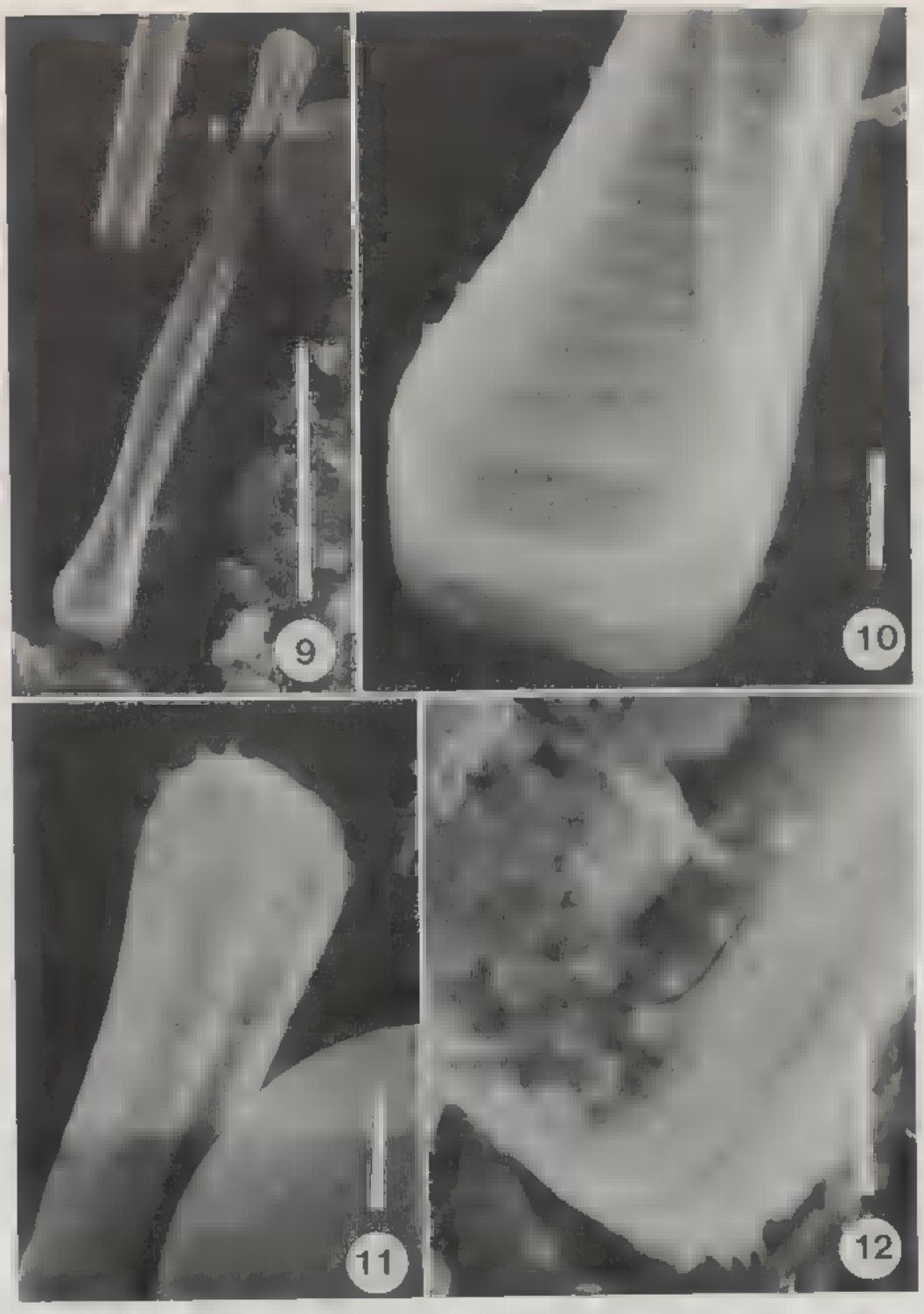
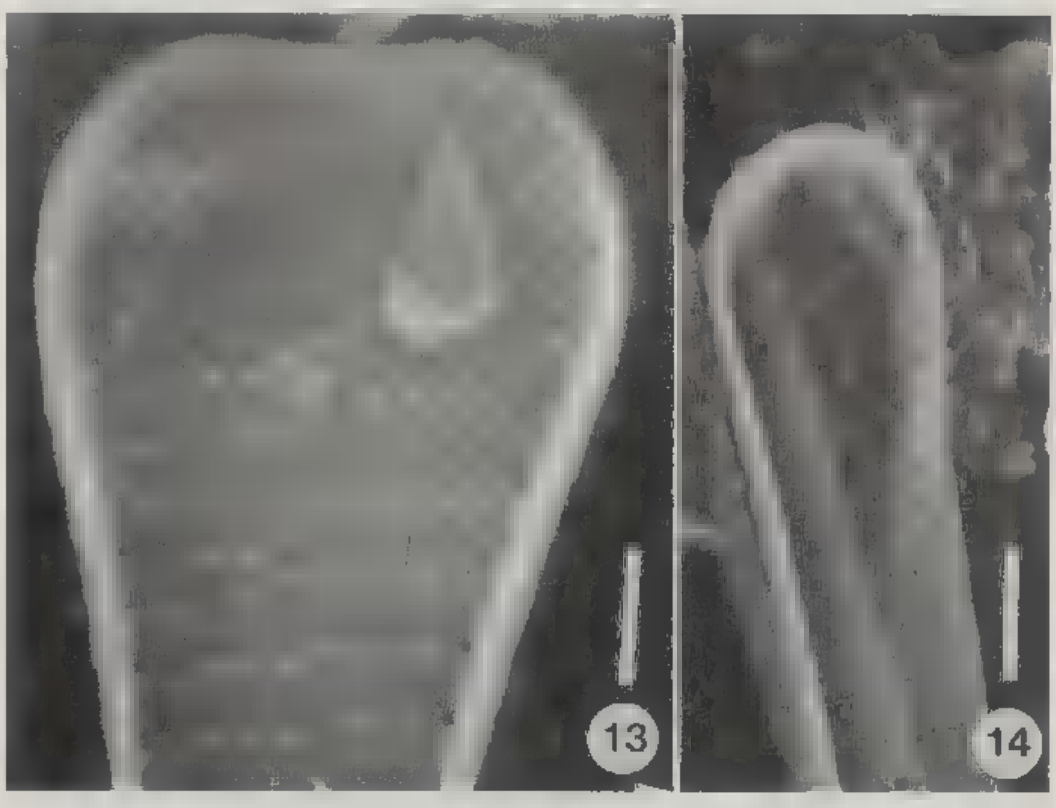


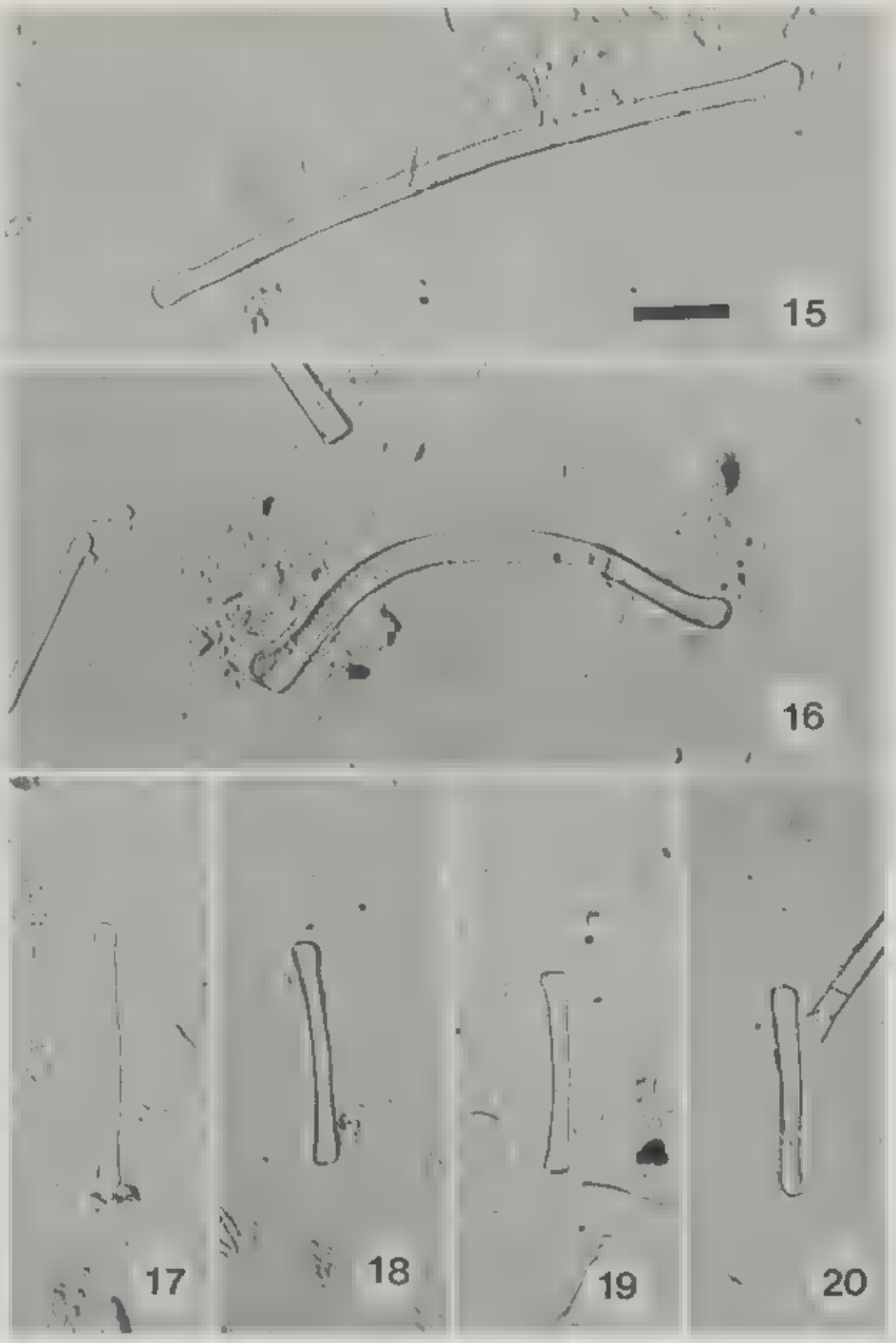
Figs 1-8. *Actinella candelabrum*, LM. Valve views. Scale bar = 10 μm.



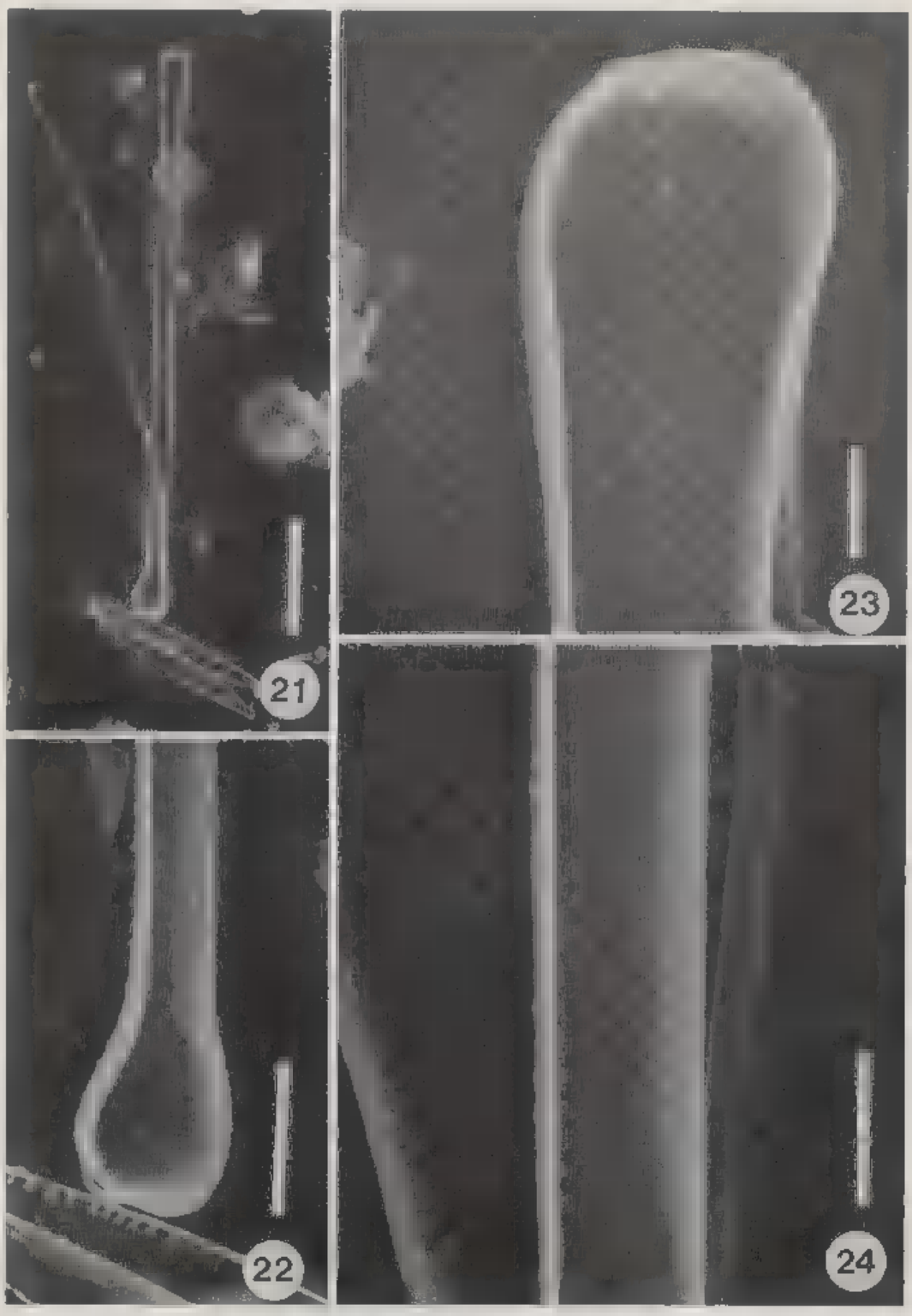
Figs 9-12. *Actinella candelabrum*. SEM. External valve views. Fig. 9. Valve view showing slight twist to valve, where the footpole is twisted out of the plane of the headpole. Scale bar = 10 µm. Fig. 10. Headpole of specimen in Fig. 9. Valve face is slightly concave, no prominent central sternum is present; a few small spines are located around the valve periphery. A small, irregular opening is present at the valve face:mantle junction. Scale bar = 1 µm. Fig. 11. Valve view of footpole of specimen in Figs 9-10, showing larger spines at valve terminus and small opening on valve face:mantle junction. Opening at footpole is on same side of valve as at headpole. Scale bar = 1 µm. Fig. 12. Girdle view of footpole showing opening extending from valve mantle onto face. Round pores and small spines are also visible. Scale bar = 1.25 µm.



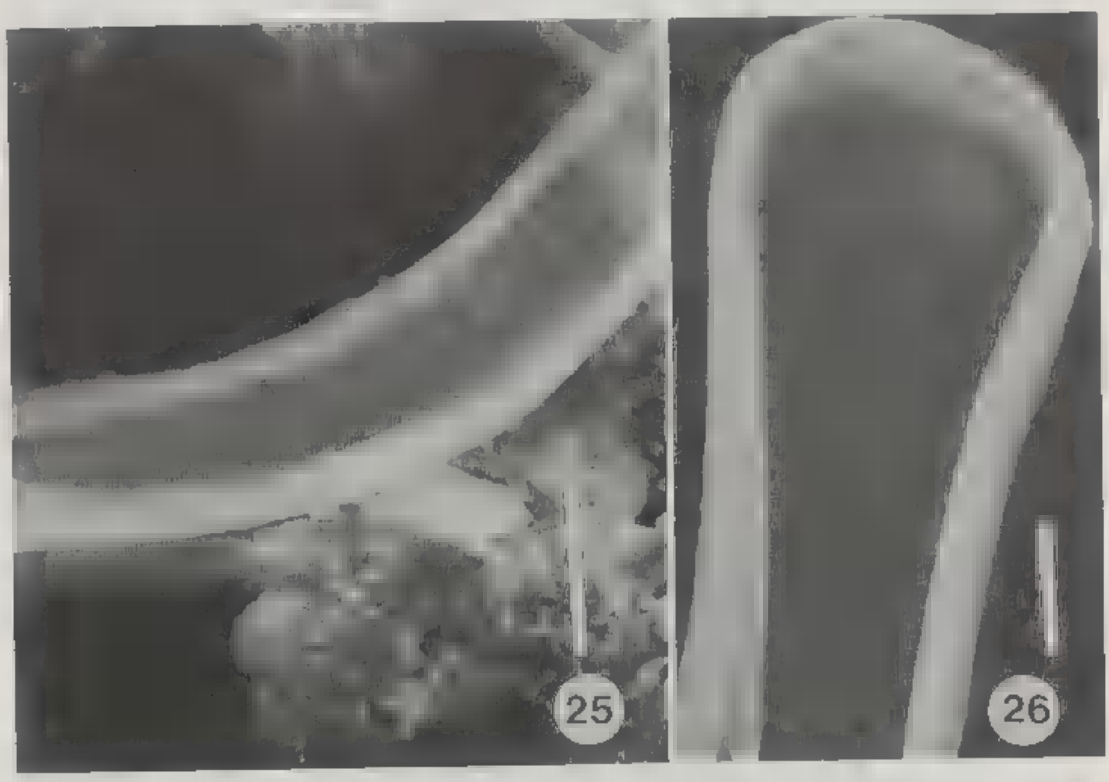
Figs 13-14. *A. candelabrum*. SEM. Internal views. Fig. 13. Headpole showing slit-like opening without helictoglossa and rimoportulae. Lack of central sternum is demonstrated. Scale bar = 1 µm. Fig. 14. Footpole showing small slit-like opening and possible rimoportula (arrow). Scale bar = 1.25 µm.



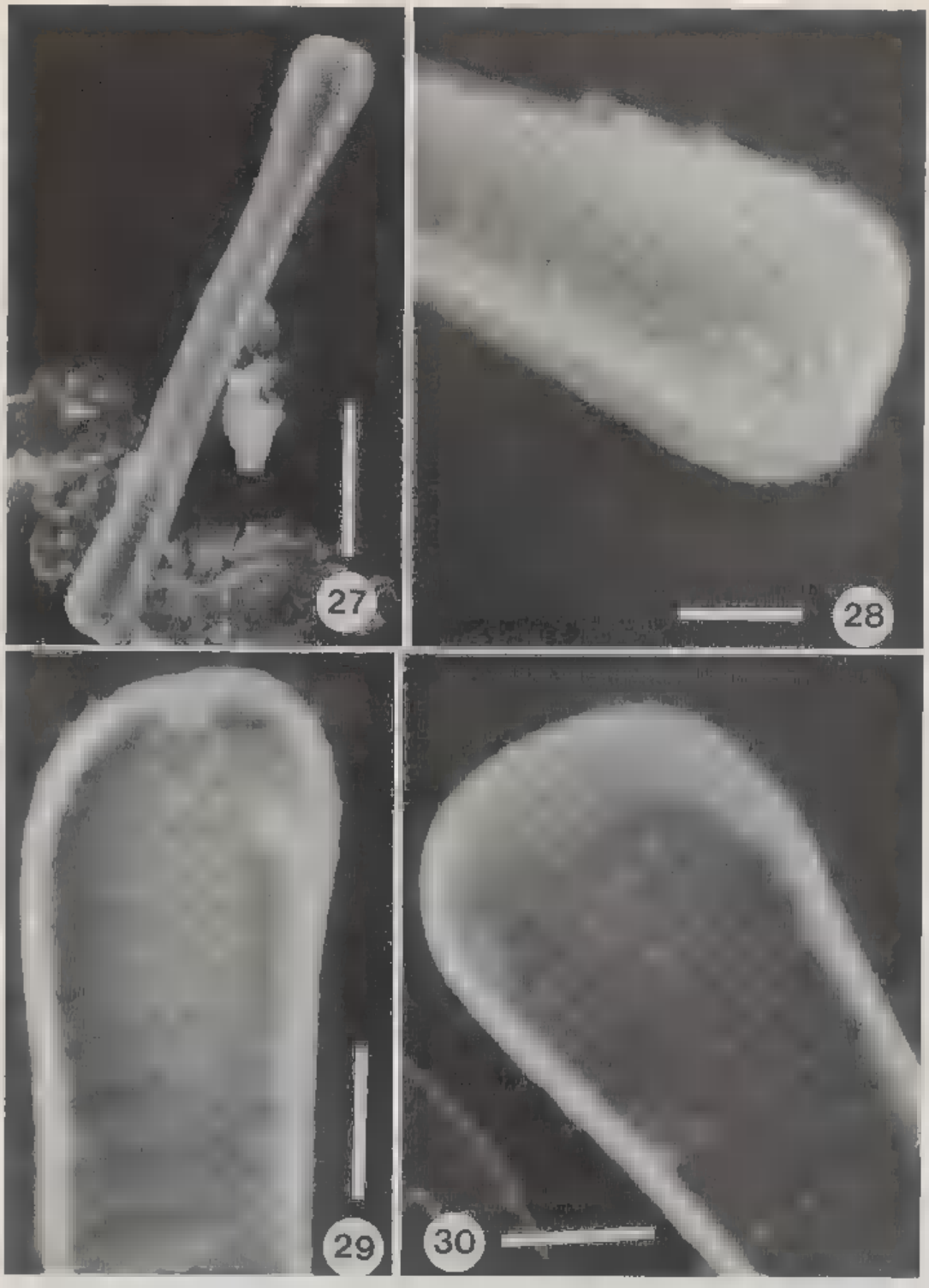
Figs 15-20. LM. Valve views. Figs 15, 16. *Actinella madagascariensis*. Figs 17-20. *Actinella bourrellyi*. Scale bar = 10 µm.



(Legends to Figs 21-24 are on p. 69).

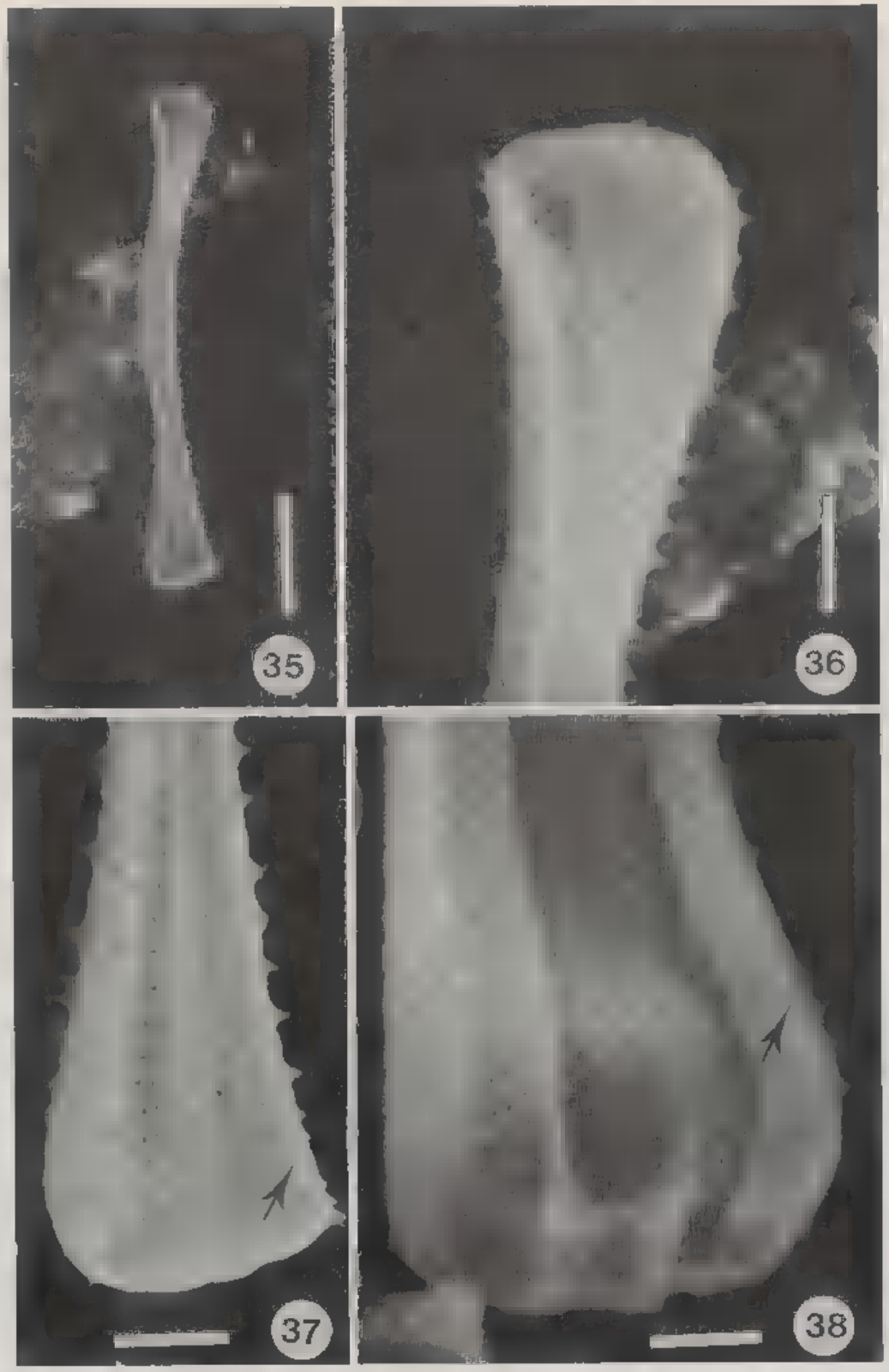


Figs 21-26. *Actinella madagascariensis*. SEM. Internal views. Fig. 21. Valve view showing overall outline of straight specimen. Scale bar = 10 µm. Fig. 22. Headpole of specimen in Fig. 21 showing shape of headpole. Scale bar = 4 µm. Fig. 23. Median part of valve from specimen in Fig. 21, showing striae traversing valve face. Scale bar = 1.25 µm. Fig. 24. Footpole showing striae radiating around pole and presence of rimoportula. Scale bar = 2 µm. Fig. 25. Central portion of curved specimen showing striae extending across valve face. Scale bar = 3 µm. Fig. 26. Footpole showing radiate nature of striae at the pole and presence of small, slit-like opening. Scale bar = 1.25 µm.

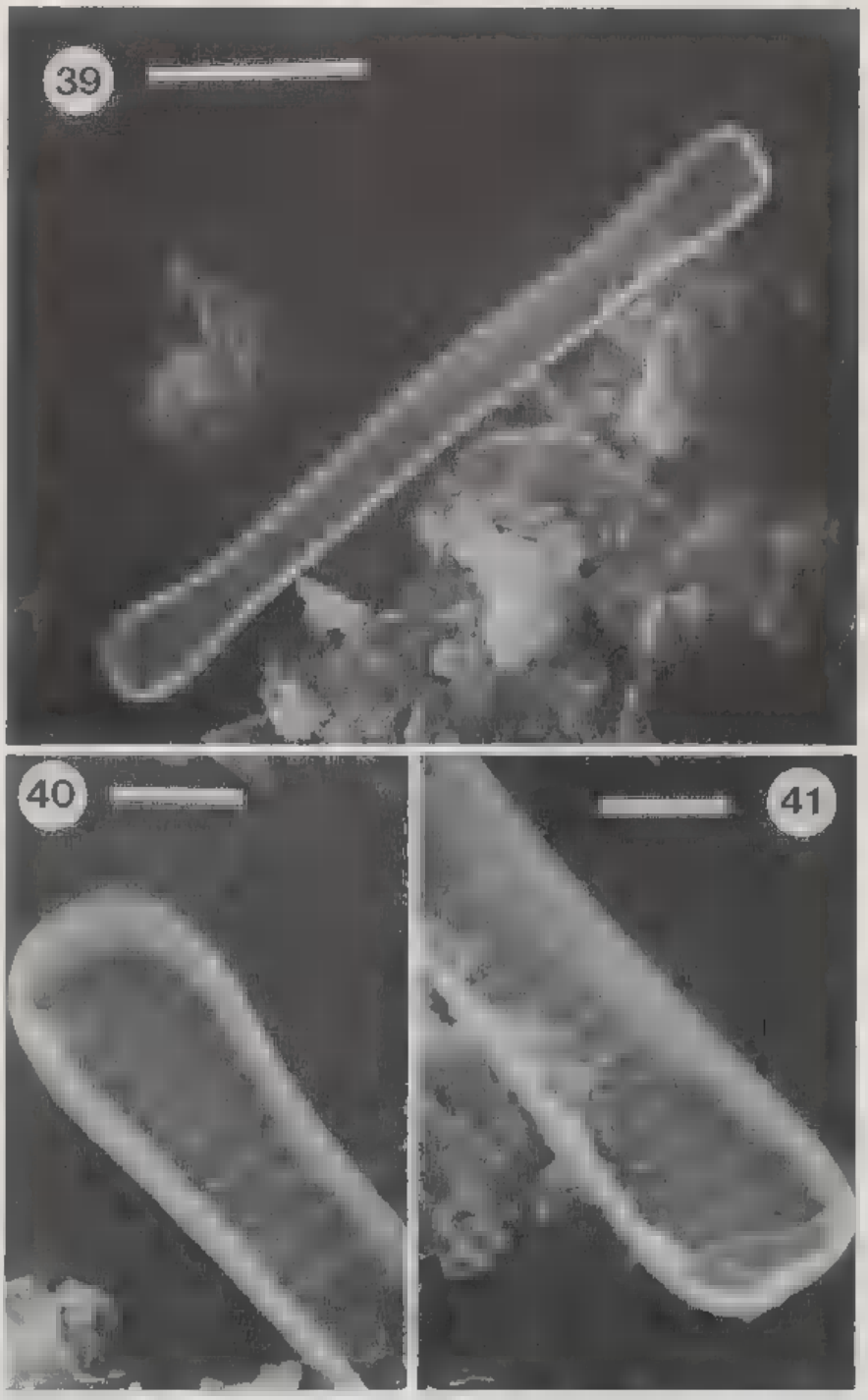


Figs 27-30. *Actinella bourrellyi*. SEM. Figs 27, 28, External views. Fig. 27. Valve view showing general valve outline, spines irregularly scattered around the periphery of the valve and distinct raphe branches at both ends of the ventral margin. Scale bar = 5 µm. Fig. 28. Footpole of specimen shown in Fig. 27, with details of the striae, small spines and raphe branch restricted mostly to the mantle but with distal raphe end curved onto the valve face. Scale bar = 1 µm. Figs 29, 30, Internal views. Fig. 29. Headpole showing raphe with small helictoglossa. Striae are shown to extend onto valve mantle. Scale bar = 1 µm. Fig. 30. Footpole with raphe ending in distinct helictoglossa and mantle bearing rimoportula. Scale bar = 1 µm.

Figs 31-34. *Actinella reviersii*. LM. Valve views. Scale bar = 10 μm.



Figs 35-38. *Actinella reviersii*. SEM. External views. Fig. 35. Girdle view showing frustule bent about apical plane. Valve is bordered by small conical spines. Scale bar = 5 µm. Fig. 36. Footpole of specimen illustrated in Fig. 35. Conical spines are shown, and several girdle bands are evident. Small raphe branches restricted to the valve mantle are evident on both valves. Scale bar = 1.25 µm. Fig. 37. Headpole of specimen shown in Figs 35, 36. One valve has raphe branch that extends from mantle to valve face while the second valve has small, irregular raphe branch that is restricted to valve mantle. Scale bar = 1.25 µm. Fig. 38. Another example of heterovalvy with respect to raphe branches; one is arched from mantle to face, the other is small, irregular and restricted to mantle. Large valvocopula has three rows of poroids. Scale bar = 1 µm.



Figs 39-41. *A. reviersii*. SEM. Internal views. Fig. 39. Valve view showing organization of striae. Scale bar = 5 µm. Fig. 40. Same specimen as Fig. 39, headpole with raphe ending in helictoglossa. Scale bar = 1.25 µm. Fig. 41. Footpole of same specimen illustrated in Figs 39, 40 showing raphe ending in helictoglossa and possible rimoportula (arrow) positioned on mantle. Scale bar = 1.25 µm.

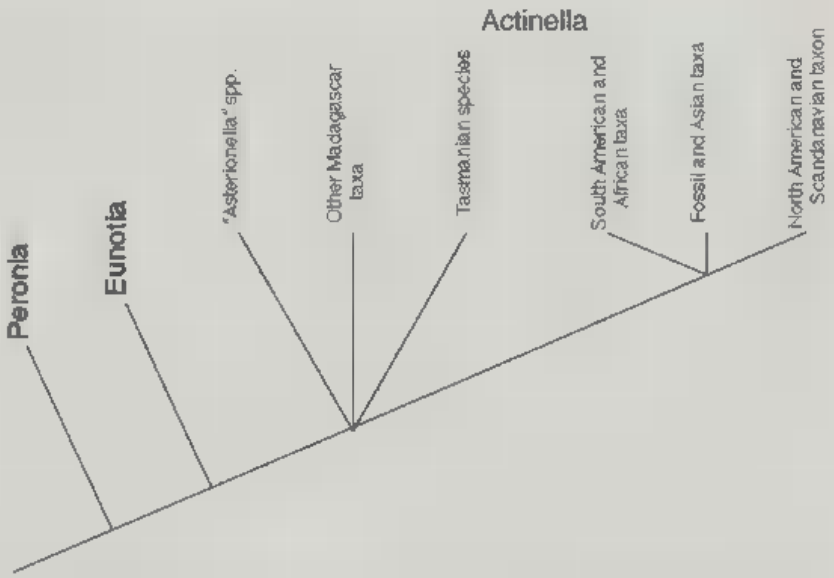


Fig. 42. Interrelationships of *Actinella* species groups. See text for details.

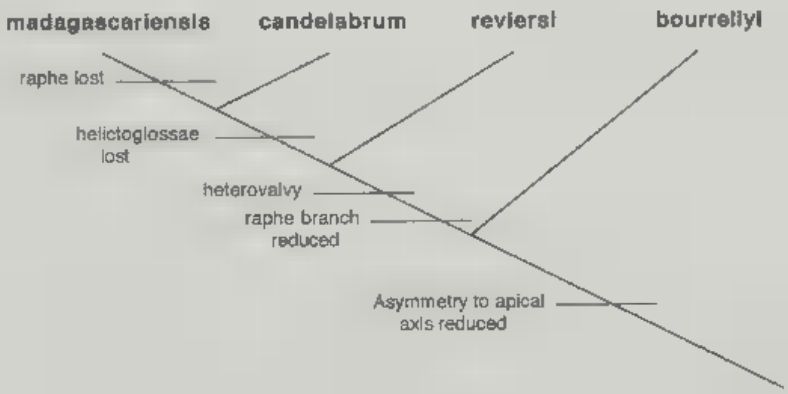


Fig. 43. Proposed set of interrelationships of *Actinella* species previously identified as *Asterionella* from Lake Tsimbazaza, Madagascar.

GOMPHONEMA PIERREBOURRELLYI SP. NOV., UN NOUVEAU *GOMPHONEMA* (BACILLARIOPHYCEAE) DU MIOCÈNE DE L'ÉQUATEUR

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ABSTRACT - A new diatom species, *Gomphonema pierrebourrellyi*, from the upper Miocene of Ecuador, is described in light and electronic microscopy. The ultrastructure of this species demonstrates that it is closely related to the other *Gomphonemata*; it has however, a unique suite of features possessing similarities with several distinct groups of gomphonemoid diatoms.

RÉSUMÉ — Une nouvelle espèce de diatomée, *Gomphonema pierrebourrellyi*, provenant du Miocène supérieur de l'Équateur, est décrite et illustrée au moyen des microscopes optique et électronique. L'ultrastructure de cette espèce montre qu'elle est étroitement alliée aux autres *Gomphonemata*; elle possède néanmoins une suite unique de caractères présentant des similitudes avec plusieurs groupes distincts des diatomées gomphonémoïdes.

KEY WORDS: diatoms, Ecuador, *Gomphonema*, new species, taxonomy, ultrastructure

INTRODUCTION

Le genre de diatomée *Gomphonema* est très vaste (plus de 1000 taxons décrits) et très commun dans les eaux douces ; cependant, nos connaissances sur la diversité, la microstructure et la répartition géographique et stratigraphique des espèces de ce genre sont limitées. De nouvelles espèces, provenant de régions lointaines et peu explorées (e.g. Lange-Bertalot, 1993 ; Kociolek & Jones, 1995), ou de régions ayant déjà fait l'objet de nombreux travaux (e.g. Krammer & Lange-Bertalot, 1993 ; Lange-Bertalot & Metzeltin, 1996 ; Kociolek & Stoermer, 1991b; Reichardt & Lange-Bertalot, 1991), continuent à être décrites. La grande diversité morphologique de ce genre a été étudiée par Kociolek et Stoermer (1991b) et Passy *et al.* (1997), ce qui suggère que tout les groupes naturels faisant partie du genre *Gomphonema* ont probablement été circonscrits. Tandis que nos connaissances des espèces récentes proviennent d'une grande variété de localités, notre compréhension des taxons fossiles dérive principalement de sites tempérés (e.g. Kociolek & Stoermer, 1989). En outre, les diatomées d'eau douce du Miocène ont fait l'objet de peu d'études en Amérique du Sud (e.g. Fourtanier *et al.*, 1993) et n'ont jamais été auparavant

décrites en Équateur. Dans cet article nous décrivons les variations morphologiques et l'ultratructure de la valve d'un nouveau *Gomphonema* fossile du Miocène supérieur de l'Équateur.

MATÉRIEL ET MÉTHODES

Cette nouvelle espèce provient d'un échantillon de diatomite qui nous a été transmis par M. Dominik Hungerbühler. Cet échantillon (n° MS 349) a été collecté le 9 août 1995, en Équateur, par M. Michael Steinmann, dans la partie Nord du Bassin de Cuenca, aux coordonnées 2°48′34,1″ S — 78°55′36,0″ W, à une altitude de 2600 m. L'échantillon provient de la Formation Turi, datée du Miocène supérieur (âge établi d'après les traces de fission des zircons par M. Steinmann) ; en fonction de sa position stratigraphique dans la Formation Turi, un âge de *ca* 8 Ma peut être extrapolé pour l'échantillon MS 349 (M. Steinmann, comm. pers., 1997).

Le matériel a été préparé en portant à ébullition l'échantillon pendant 20 mn dans de l'eau oxygénée (10 %), puis en le rinçant à l'eau distillée. Une fraction du résidu a été montée entre lame et lamelle à l'aide de Naphrax pour les observations en microscopie optique, ou déposée sur une lamelle de verre, séchée et recouverte d'une couche d'or de 20 nm pour les observations en microscopie électronique à balayage (MEB). Les observations au microscope optique ont été effectuées à l'aide d'un Leica DMRB. Les observations au MEB ont été réalisées à l'aide d'un Hitachi S-520.

RÉSULTATS

***Gomphonema pierrebourrellyi* Fourtanier *et* Kociolek, sp. nov.**
(Figs 1-18)

Description. Valves largement à étroitement anguleuses, à pôles pointus, les spécimens les plus petits lancéolés-clavés avec le pôle supérieur légèrement prolongé. Longueur 16-57 µm, largeur 4,5-10,0 µm. Stries ponctuées, parallèles au centre, radiées vers le pôle inférieur, radiées à parallèles vers le pôle supérieur, stries médianes plus espacées, 10-13/10 µm au centre, 15-20/10 µm aux pôles. Aire axiale linéaire, quelque peu élargie au centre de la valve ; aire centrale irrégulière, définie par une ou plusieurs stries prolongées sur l'un ou les deux cotés de l'aire axiale, avec un ou deux petits stigmas et un ou plusieurs pores isolés. Raphé latéral, légèrement ondulé, terminaisons internes proximales du raphé distinctes et détournées vers le stigma. Champ de pores apical indistinct. Septa non visibles.

Diagnose latine. *Valvae late ad anguste angulatae, polis acutis ; specimina parviora lanceolata-clavata polo capitali leviter protracto. Longitudo 16-57 µm. Latitudo 4.5-10.0 µm. Striae punctatae, parallelae ad centrum, radiatae polum pedalem versus, radiatae ad parallelae polum capitalem versus, striae mediae dissitiores, 10-13/10 µm ad centrum, 15-20/10 µm ad polos. Area axialis linearis, aliquantum lata ad centrum valvae ; area centralis irregularis, finita per unam ad aliquot strias elongatas in uno vel ambobus lateribus*

areae axialis, uno vel duobus stigmatibus parvis et uno vel aliquot punctis sejunctis. Raphe lateralis, leviter undulata, extremitatibus internis proximalibus raphes manifestis et stigma versus deflexis. Area porellibus apicalis non manifesta. Septa non visibilia.

Holotype. Spécimen illustré Fig. 5, cerclé sur la lame n° 219054 déposée à la Collection de Diatomées de la California Academy of Sciences (San Francisco).
Localité type. Bassin de Cuenca (Équateur), Formation Turi (Miocène supérieur) ; coordonnées: 2°48′34,1″ S — 78°55′36,0″ W ; altitude : 2600 m.

Etymologie. Espèce nommée en l'honneur du Professeur Pierre Bourrelly, l'un des plus remarquables phycologues du vingtième siècle.

Observations ■ MEB. En face externe, les stries sont composées d'aréoles en forme de C, de S, ou de formes irrégulières (Figs 9-12). Au centre de la valve, les extrémités proximales du raphé ne sont pas dilatées (Fig. 15). Au pôle supérieur, l'extrémité distale du raphé est déviée sur la face valvaire puis se prolonge sur le manteau ; les stries sont encore présentes autour du pôle supérieur mais sont limitées au manteau (Figs 9-10). Au pôle inférieur, l'extrémité distale du raphé est détournée sur la face valvaire, puis traverse le champ de pores apical (CPA). Les pores du CPA sont arrondis et presque (Fig. 11) ou totalement (Fig. 12) limités au manteau.

En face interne, le nodule central est légèrement surélevé et porte les extrémités proximales recourbées du raphé ainsi que l'ouverture du stigma. L'ouverture du stigma est arrondie ou légèrement allongée (Fig. 14). L'hélictoglosse du pôle supérieur (Fig. 16) et celle du pôle inférieur (Fig. 17) sont légèrement décalées par rapport à l'ouverture du raphé. Un pseudoseptum est présent à chaque pôle.

DISCUSSION

Gomphonema pierrebourrellyi ressemble à *G. gracile* Ehrenberg par le contour anguleux des valves et leurs dimensions, mais il en diffère par son aire axiale large, les terminaisons proximales du raphé distinctement recourbées sur la face interne, et l'orientation des stries. La variabilité de forme chez *Gomphonema pierrebourrellyi*, où les spécimens les plus petits deviennent subcapités, est unique parmi les *Gomphonemata* anguleux.

Gomphonema pierrebourrellyi est dominant dans l'échantillon sur lequel cette étude est basée. Sont aussi présents dans l'association *Cocconeis* sp., *Amphora* sp. ainsi que d'autres espèces benthiques suggérant un habitat lacustre en fond ou en bordure de lac. La plupart des espèces de l'association semblent n'avoir jamais été décrites, aussi il est impossible de préciser davantage l'environnement attaché à cet échantillon faute des données écologiques nécessaires.

L'ultrastructure de la valve de *Gomphonema pierrebourrellyi* démontre qu'il est étroitement allié aux autres espèces de *Gomphonema*. Les occlusions partielles des aréoles sur la face externe de la valve (Kociolek & Stoermer, 1991b) de *G. pierrebourrellyi* sont similaires à celles des diatomées gomphonémoïdes d'eau douce (e.g. Dawson, 1972 ; Krammer & Lange-Bertalot, 1986, 1991 ; Round *et al.*, 1990). Par la forme des ouvertures externes des aréoles, cette nouvelle espèce semble plus proche des membres du groupe Herculeana du genre *Gomphoneis* (*sensu* Kociolek & Stoermer, 1988b, 1993a) qu'au

groupe *Gomphonema* sensu stricto (e.g. *G. acuminatum* Ehrenberg, *G. parvulum* (Kütz.) Kutz. ; Kociolek & Stoermer, 1991b ; Dawson, 1972).

L'ouverture interne du stigma de *G. pierrebourrellyi* est arrondie alors qu'elle est en forme de fente chez la plupart des *Gomphonematata* (e.g. Kociolek & Stoermer, 1991a, 1991b ; Lange-Bertalot, 1993). L'ouverture interne du stigma de *Gomphonema clevei* Fricke cependant est arrondie (Kociolek & Stoermer, 1991a). L'ouverture interne arrondie du stigma de *G. pierrebourrellyi* peut être l'expression réelle de cette structure, elle peut toutefois être due à la dissolution partielle de la terminaison de l'ouverture (laissant une apparence plus arrondie, tubulaire au stigma).

Chez *Reimeria* Kociolek & Stoermer l'ouverture interne du stigma est également arrondie ou largement elliptique (Kociolek & Stoermer, 1987). En dépit de la similitude de l'ouverture du stigma entre les espèces du genre *Reimeria* et certains *Gomphonema*, l'opinion exprimée par Krammer (1982) puis par Round *et al.* (1990), Lange-Bertalot & Metzeltin (1996) et Cox (1996) que *Reimeria* est un membre de la lignée gomphonémoïde n'est pas acceptable. La présence d'une symétrie cymbelloïde et l'absence des synapomorphies (caractères dérivés communs) qui sont indicatrices de la lignée gomphonémoïde des diatomées d'eau douce, soutiennent le placement de *Reimeria* dans la lignée cymbelloïde des diatomées d'eau douce (Kociolek & Stoermer, 1988a).

La suite unique de caractères observés chez *Gomphonema pierrebourrellyi*, possédant des similarités avec plusieurs groupes distincts des diatomées gomphonemoïdes, souligne l'importance et la nécessité de continuer les études systématiques dans cette lignée extrêmement variable et intéressante des diatomées d'eau douce.

REMERCIEMENTS — Nous sommes reconnaissants à Messieurs M. Steinmann et D. Hungerbühler (ETH Zurich) de nous avoir procuré cet échantillon. Nous remercions Monsieur B. de Reviers de nous permettre d'apporter cette contribution au fascicule dédié à la mémoire de l'éminent Professeur Pierre Bourrelly.

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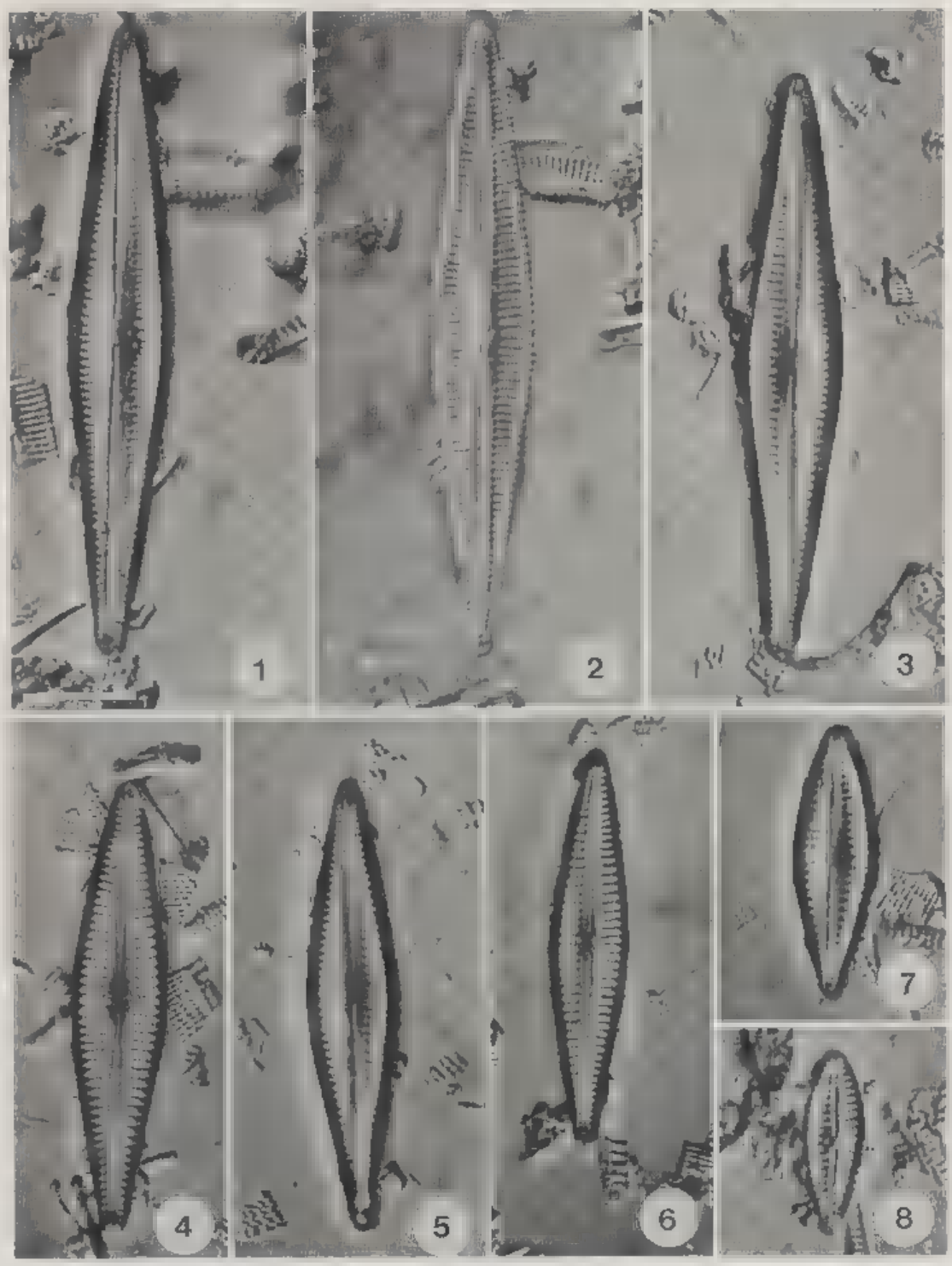
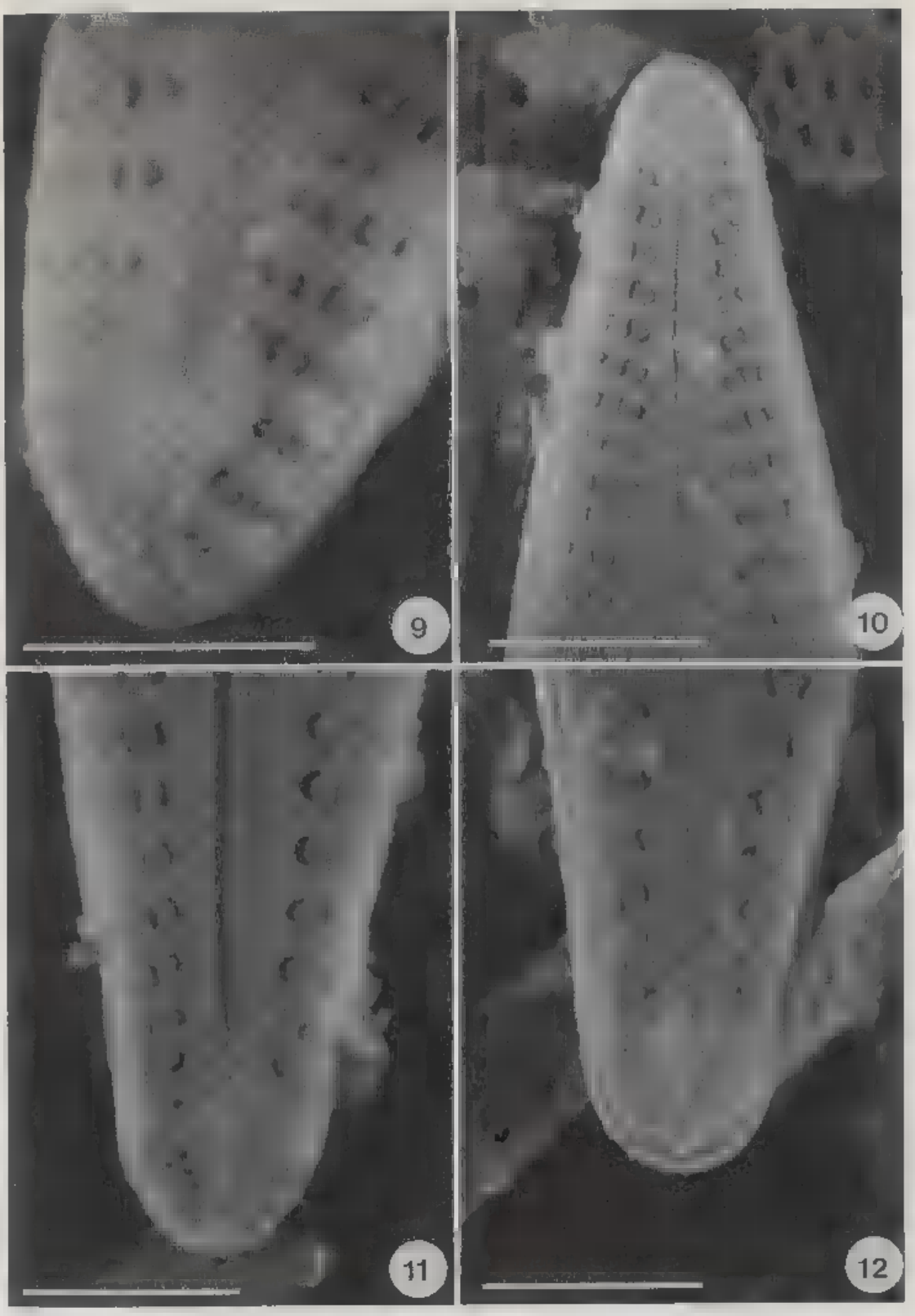
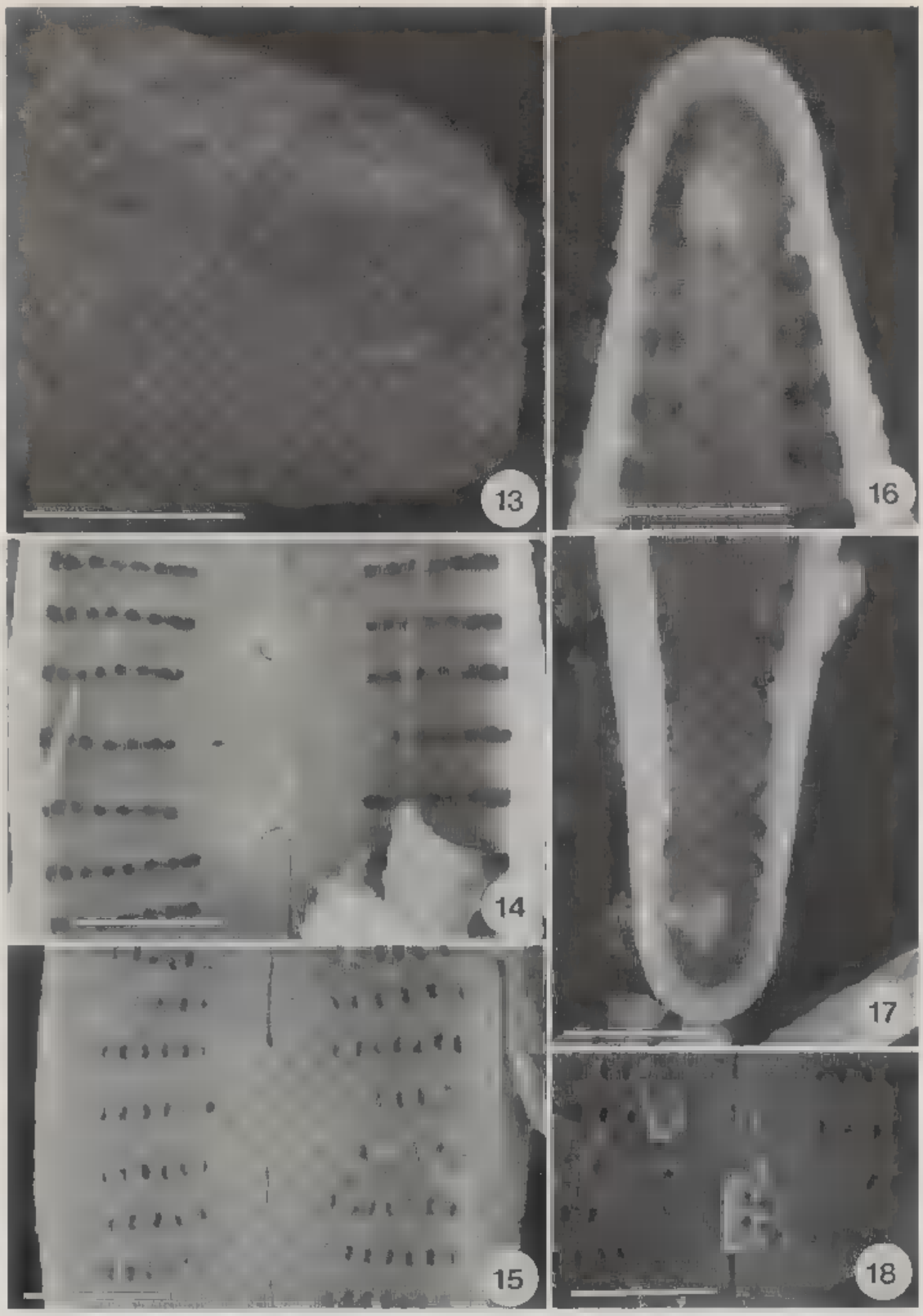


Figs 1-8. *Gomphonema pierrebourrellyi* sp. nov., microscopie optique. Échelle = 10 µm. Vues valvaires montrant la variabilité des tailles et des formes. Figs 1-2. Même spécimen à deux mises au point différentes. Fig. 5. Holotype. Tous les spécimens photographiés appartiennent à la lame type.



Figs 9-12. *Gomphonema pierrebourrellyi* sp. nov., microscopie électronique (MEB). Échelles = 2 µm. Figs 9-10. Vues externes du pôle supérieur montrant les occlusions partielles des aréoles et les extrémités distales du raphé . Figs 11-12. Vues externes du pôle inférieur montrant le champ de pores apical traversé par l'extrémité distale du raphé.



Figs 13-18. *Gomphonema pierrebourrellyi* sp. nov., microscopie électronique (MEB). Échelles = 2 µm. Fig. 13. Vue connective. Fig. 14. Vue interne, partie centrale de la valve montrant le nodule central avec l'ouverture du stigma et les extrémités recourbées du raphé. Figs 15, 18. Vues externes, partie centrale de la valve. Le spécimen illustré Fig. 15 a un stigma unique, le spécimen illustré Fig. 18 a deux stigmas. Fig. 16. Vue interne du pôle supérieur montrant l'hélictoglosse et l'extrémité distale du raphé. Fig. 17. Vue interne du pôle inférieur.

NEW AND CONFUSED SPECIES IN THE GENUS *NAVICULA* (BACILLARIOPHYCEAE) AND THE CONSEQUENCES OF RESTRICTIVE GENERIC CIRCUMSCRIPTION

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ABSTRACT — Electron microscopic investigations indicate that there are serious taxonomic problems with some taxa which, at a first glance seem to fit the restricted generic diagnosis of *Navicula*. Even if these taxa apparently resemble *Navicula* as typified by Patrick (1959; cf. Cox, 1979; Round *et al.*, 1990) under LM. SEM examination reveals several important morphological differences. Six different morphological groups have been distinguished within the genus *Navicula*. They differ with respect to stria structure, lineola shape, external central raphe endings, terminal raphe endings, position of the internal raphe fissures and cingulum structure. It remains to be decided whether subgenera should be created within the genus *Navicula*, or whether particular subgroups should be raised to generic rank. We think that future studies will provide further evidence of the heterogeneity of the genus *Navicula*. However, *N. bourrellyivera*, *N. hanseatica*, *N. rolandii*, *N. vaneei* and *N. witkowskii* are described here as new species which strictly conform to *Navicula* as typified by *N. tripunctata*.

RÉSUMÉ — Les études en microscopie électronique indiquent que les taxons qui, au premier abord, semblent correspondre à la diagnose d'une vision restreinte du genre *Navicula*, posent, en fait, des problèmes taxinomiques sérieux. Même si ces taxons ressemblent au genre *Navicula* tel qu'il fut typifié par Patrick (1959; cf. Cox, 1979; Round *et al.*, 1990) en microscopie optique, l'examen au microscope électronique à balayage révèle des différences morphologiques importantes. Sept groupes morphologiques différents ont été distingués au sein du genre *Navicula*. Ils diffèrent par la structure des striae, la forme des lineolae, les extrémités du raphe central, les extémités du raphe terminal, la position des fissures du raphe interne et la structure du cingulum. Il reste à décider si des sous-genres doivent être créés au sein du genre *Navicula*, ou si des sous-groupes particuliers doivent etre élevés au rang de genre. Nous pensons que des études futures apporteront des preuves supplémentaires de l'hétérogénéité du genre *Navicula*. Cependant, *N. bourrellyivera*, *N. hanseatica*, *N. rolandii*, *N. vaneei* et *N. witkowskii* sont décrits ici comme des espèces nouvelles strictement conformes au genre *Navicula* tel qu'il a été typifié par *N. tripunctata*. (Traduit par la Rédaction)

KEY WORDS: Bacillariophyceae, diatoms, *Navicula*, Naviculaceae, new species, microalgae, morphology, taxonomy.

INTRODUCTION

The circumscription of *Navicula* Bory (Naviculaceae, Bacillariophyceae) has varied greatly. In addition to the Section Lineolatae, *Navicula* has included several other sections and an undetermined number of taxa which do not neccessarily possess naviculoid valve outlines (e.g. Cleve, 1894-1895; Peragallo & Peragallo, 1897-1908; Hustedt, 1927-1966; Krammer & Lange-Bertalot, 1986). With establishment of the segregate genera *Haslea* (Simonsen, 1974), *Proschkinia* (Karayeva, 1978b) and *Lyrella* (Karayeva, 1978a), however, circumscription of *Navicula* became more restricted. This process has continued in two ways: either by creating new genera e.g. *Parlibellus* by Cox (1988); or by resurrecting genera whose member species were placed in *Navicula* [e.g. *Placoneis* Mereschkowsky (Cox, 1987a) and *Sellaphora* Mereschkowsky (Mann, 1989)]. Round *et al.*, (1990); Lange-Bertalot & Metzeltin (1996), Lange-Bertalot *et al.*, (1996), and Witkowski *et al.*, (1997) have provided further refinement. Patrick (1959) typified *Navicula* with *Navicula tripunctata* (O.F. Müller) Bory and Cox (1979) provided a detailed description of *N. tripunctata* and emended the generic circumscription of *Navicula*. Subsequently Round *et al.* (1990) and Round (1996) strongly recommended that *Navicula* be used in a restricted sense *i.e.* only for taxa belonging to the former Section Lineolatae, which includes *N. tripunctata*.

Recent studies, however, reveal that even this concept of *Navicula* includes a heterogeneous assemblage of taxa, as already suggested by Cox (1979). Chloroplast structure and valve morphology as observed in LM are of little significance. Only SEM studies allow particular subgroups within *Navicula* to be distinguished. Based upon valve morphology Lange-Bertalot *et al.* (1996) segregated *Navicula capitata* Ehrenberg and related taxa into a new genus, *Hippodonta*.

Over the last few years strong opposition arose against placing taxa in the genus *Navicula* which do not fit the diagnosis of the former Section Lineolatae. Since the publication of Round *et al.* (1990) the genus *Navicula* has become particularly ambiguous (*i.e. Navicula sensu stricto* and *sensu lato*). In *Navicula sensu stricto* taxa with the following characteristics are included: boat shaped valves, lineolate striae, central external raphe endings simple, apical endings hooked to one side, internal raphe slit running obliquely in raised ribs and without visible central pores and two lateral plastids (Cox, 1979; Round *et al.*, 1990; Round, 1996). *Navicula sensu lato* includes groups of species which do not conform with the above criteria. A provisional solution for "homeless" taxa was recently proposed by Lange-Bertalot *in* Lange-Bertalot & Moser (1994) who established *Naviculadicta* for taxa which belong neither in *Navicula sensu stricto* nor in other established genera. This approach could be used until an adequate classification accomodates all "homeless" taxa.

In the present paper results of our studies on the diatom genus *Navicula* are introduced. In this paper, which originates from a project dealing with diatom flora of the marine littoral, our attention was focused on taxa which are routinely identified as representatives of *Navicula* in the light microscope. It was our intention to show that *Navicula*, in the sense of the former Section Lineolatae with *N. tripunctata* as the type is still heterogenous genus.

MATERIAL AND METHODS

Predominantly surface sediment samples from brackish-water and marine littoral localities from various geographic regions were studied (Table 1). The surface sediments from the Gulf od Gdańsk and Puck Bay (Poland) were irregularly sampled during 1991-1993, but at least once a quater (Stachura & Witkowski, in press). During 1993 selected stations along the shore of the Gulf of Gdańsk and Puck Bay including salt marsh area were sampled at monthly intervals. Samples from the Mecklenburg Bay originated from 13 cores (up to 6 m long). In all, several hundreds of samples from around the world were studied, but for this particular paper only 26 sites were chosen. Samples were selected to cover all possible environmental conditions with respect to salinity, climate and substrate.

Table. 1. Samples studied, localities and collections.

No.	Sampling site	Geographic region	Substrate	Country	Collection
1.	Władysławowo	Puck Bay Baltic Sea	salt marsh	Poland	Witkowski
2.	Gulf of Gdańsk	Baltic Sea	surface sediments	Poland	Witkowski
3.	Puck Bay	Baltic Sea	surface sediments	Poland	Witkowski
4.	Mecklenburg Bay	Baltic Sea	fossil sediments	Danmark/ Germany	Witkowski
5.	Kattegat	Baltic Sea	surface sediments	Danmark	Witkowski
6.	Bear Island	Barents Sea	tidal flat sediments	Norway	Witkowski/ Lange-Bertalot
7.	Franz Joseph Land	Arctic Ocean	*Laminaria* sp.	Russia	Witkowski
8.	fjord near Narvik	Norwegian Sea	sediment	Norway	Reichardt
9.	Weser River		sediment	Germany	Lange-Bertalot
10.	Reckerta River	NW Germany	sediment	Germany	Lange-Bertalot
11.	mouth of the River Weser	North Sea Bremerhaven	tidal flat	Germany	Brockmann
12.	La Rochelle	Atlantic Ocean	sediment	France	Lange-Bertalot
13.	Vila Franco da Campo	Atlantic Ocean Azores	algal mat rock pool	Portugal	Lange-Bertalot
14.	Mississippi Delta	Gulf of Mexico	sand	USA	Lange-Bertalot
15.	Campeche	Gulf of Mexico	sediment	Mexico	Lange-Bertalot
16.	Lake Mir	Mediterranean	sediment	Croatia	Lange-Bertalot

17.	Coast of Crete	Mediterranean	sediment	Greece	Reichardt
18.	Eilat	Red Sea	sediment	Israel	Reichardt
19.	Dar-es-Salam	Indian Ocean	sediment	Tanzania	Moeller (Copenhagen)
20.	Tonga harbour	Indian Ocean	harbour	Tanzania	Reichardt
21.	Coast of Kenya	Indian Ocean	sediment	Kenya	Reichardt
22.	Qurum Beach	Gulf of Oman	calcareous sand	Oman	Lange-Bertalot
23.	St. Gilles-les-Bains	Indian Ocean	algal mat	La Réunion	Reichardt
24.	Fiji Islands	Pacific Ocean	sediment	Fiji	Foged (Copenhagen)
25.	Kok-tao Island	Pacific Ocean	sand	Thailand	Lange-Bertalot
26.	Drake's Bay	California	sand	USA	Reichardt

The samples were cleaned by boiling in concentrated HCl, washed several times with distilled water, and boiled in H_2SO_4 with small amounts of KNO_3 added at *ca* 15 min. intervals. Other samples were boiled in concentrated hydrogen peroxide in order to remove organic matter. After washing several times with distilled water, the samples were dried onto coverglasses. Coverglasses were attached to slides with Naphrax.

Light microscopic studies were carried out by means of a Leitz Diaplan microscope with 63/1.4 PlanAPO oil immersion objective. SEM studies were performed by means of a Hitachi S 4500. All the SEM stubs are deposited at the Botanical Institute of J.-W. Geothe University, Frankfurt am Main in collection Lange-Bertalot.

RESULTS

The results of our study are arranged into seven sections. In six sections particular groups of taxa which shows consistent characteristics are introduced. In the seventh one a mixed group of taxa is presented. There is no doubt that they differ from *Navicula sensu stricto*, but so far they have only been found as single entities so that, at present, no grouping is possible. Newly described taxa are compared with other related species of *Navicula sensu stricto* (Table 2). Finally each of the six groups is presented by listing the most important characters (Table 3).

Navicula sensu stricto (Figs 1-14, 23-32, 35-38, 88-93)

Navicula sensu stricto encompasses *ca* 150 species inhabiting freshwater and *ca* 50 ones living in brackish water and the marine environment. Our observations indicate that there are still numerous undescribed taxa, belonging to *Navicula sensu stricto* inhabiting marine littoral. All the taxa included in *Navicula sensu stricto* possess characters found in *N. tripunctata*. The most important features of *Navicula sensu stricto* are given in Table 2. They are characterized by striae composed of numerous lineolae, simple central external raphe endings, hooked apical raphe endings, internal raphe fissure running obliquely in raised ribs and cingulum composed of open bands. Here five new taxa are being described. Three of the following new species belong to an apparently closely related group of taxa

living predominantly in brackish waters e.g. *N. slesvicensis* Grunow, *N. meniscus* Schumann, *N. rhynchotella* Lange-Bertalot, *N. peregrina* (Ehr.) Kützing and *N. kefvingensis* (Ehr.) Kützing. The remaining two taxa *N. witkowskii* and *N. rolandii* are related to *N. salinarum* Grunow. For at least three of the new taxa, single specimens were already depicted by various authors (e.g. Germain, 1981; Krammer & Lange-Bertalot, 1986) but were lumped within the more prominent species, for example *N. rhynchocephala* Kützing. Without a doubt, all of these taxa belong to *Navicula sensu stricto* showing all diagnostic characteristics with *N. tripunctata*.

Navicula bourrellyivera **Lange-Bertalot, Witkowski & Stachura sp. nov. Figs 1-6.**
Type: Praep. No. Eu-PL 72 in Coll. Lange-Bertalot, Botanical Institute University of Frankfurt
Type locality: Salt marsh, Władysławowo, Puck Bay, Poland (*leg.* A. Witkowski, 1993).

Latin diagnosis. *Valvae lanceolatae vel modice lineari-lanceolatae apicibus cuneatis plusminusve longe protractis ad extremum acutius ad obtusius rotundatis. Longitudo 30-55 µm, latitudo 10-12 µm. Raphe filiformis ad modice lateralem poris centralibus distincte signatis. Area axialis angusta, area centralis fere parva ad modice amplam, transverse rectangularis vel lanceolata. Striae transapicales radiantes ad apices versus convergentes, 9-11 in 10 µm, lineolae crassae apparentes, 20-21 in 10 µm.*

Etymology. This species is dedicated to our late colleague and exemplary international phycologist Pierre Bourrelly. The epithet *bourellyivera* was chosen because the combination *Navicula bourrellyi* Manguin (Manguin, 1960) is in use for a taxon which does not belong to the genus *Navicula*, but must be transferred to another, as yet undefined genus.

Diagnosis. Valves lanceolate to moderately linear-lanceolate with moderately acutely rounded, more or less protracted apices, 30-55 µm long, 10-11 µm wide. Raphe filiform to moderately lateral, external central raphe endings distinctly expanded, terminal raphe endings strongly curved in the same direction. Axial area linear, narrow, central area somewhat variable in shape, transversely rectangular to lanceolate, usually asymmetrical. Transapical striae radiate in the middle, becoming slightly convergent towards apices, 9-11 in 10 µm. Striae composed of distinct lineolae, 20-21 in 10 µm.

Distribution and ecology. It occurs in brackish waters of the Baltic and North Sea. Usually infrequent but sometimes abundant in local populations. This species also has been encountered infrequently and with few individuals in freshwater, in particular rivers under eutrophic conditions and with higher conductivity.

N. bourrellyivera might have been overlooked in the past or confused with *Navicula rhynchocephala*. It belongs to ■ group of taxa which are closely related morphologically (see below) and live under lower to higher brackish water conditions. Among this group *N. hanseatica* Lange-Bertalot & Stachura has the most similar combination of characteristics in common with *N. bourrellyivera*. However, the taxa are easy to distinguish from one another if associated in the same samples, as is the case in the type locality. They differ in valve breadth, number of striae in 10 µm and shape of the central area (Table 2).

Navicula hanseatica Lange-Bertalot & Stachura sp. nov. Figs 23-27
Type: Praep. No. Eu-PL 72 in Coll. Lange-Bertalot, Botanical Institute University of Frankfurt
Type locality: Salt meadow, Władysławowo, Puck Bay, Poland (*leg.* A. Witkowski, 1993).

Latin diagnosis. *Valvae lanceolatae apicibus quoad individua minutissima simpliciter cuneatis quoad individua media ad maxima plusminusve longe protractis ad extremum acute*

rotundatis nonumquam capitatis vel subcapitatis. Longitudo 30-70 µm, latitudo 12-15 µm. Fissurae raphis paulo laterales poris centralibus crasse signatis. Area axialis angusta, area centralis ampla plerumque transverse rectangulata. Striae transapicales radiantes ad apices versus convergentes, 8-9 in 10 µm. Lineolae comparate conspicue crassae apparentes, 20-21 in 10 µm.

Diagnosis. Valves lanceolate with protracted and acutely rounded ends in middle sized and longer specimens, and simply cuneate ends only in the smallest specimens. Ends never capitate or subcapitate. Length 30-70 µm, breadth 12-15 µm. Raphe fissures moderately lateral with distinctly expanded central pores. Axial area narrow, central area large mostly transversely expanded. Striae radiate but at the ends convergent, 8-9 in 10 µm. Lineolae comparatively very coarse, 20-21 in 10 µm. SEM: Central raphe fissures endings outside into large central pores with hooked processes (similar but less distinctly than in *Navicula peregrina*). Valve face flat, the sternum does not form an elevated rib outside (Figs 37-38).

Etymology. The name of the new species is derived from "Hanse" ■ powerful, medieval trade organization which existed in the Baltic Sea region.

Distribution and ecology. It is frequent to abundant in brackish waters of the marine littoral in Europe and river estuaries.

New species is identical with *N. rhynchocephala* var. *amphiceros sensu* Germain (1981, fig. 69: 2) *N. rhynchotella* (cf. Lange-Bertalot 1993) is similar in many characteristics, morphological and ecological, however, the valves are not tapering to the ends but are protracted more abruptly in all stages of the cell cycle. Furthermore the smallest valves have no cuneate ends (Table 2).

Navicula rolandii Wunsam, Witkowski & Lange-Bertalot sp. nov. Figs 88-93.
Type: Praep. No. E-Lok 103 in Coll. Lange-Bertalot, Botanical Institute University of Frankfurt.
Type locality: Lake Mir, Croatia, the Mediterranean, (*leg.* S. Wunsam, 1995).

Latin diagnosis. *Valvae lineari-ellipticae apicibus curte cuneatis ad extremum fere obtuse nec distincte acute rotundatis, 30-47 µm longae, 10-11 µm latae. Raphe filiformis ad mediam versus declinata in poros centrales incrassatos dense positos inter se. Fissurae terminales curte hamatae unilateraliter ut generaliter in Navicula. Area axialis angustissima, area centralis parva fere variabilis ad instar. Striae transapicales irregulariter undulatae conspicue radiantes ad apices versus parallelae vix convergentes, in media nonnullae striae abbreviatae interpositae apparentes, 10-11 in 10 µm. Lineolae difficiliter discernandae, 35 in 10 µm.*

Diagnosis. Valves linear-elliptical with more or less cuneate, rather obtusely than acutely rounded apices, 34-47 µm long, 10-11 µm wide. Raphe filiform, external central raphe endings distinct, very close to each other. Terminal raphe endings somewhat declined and strongly curved to the same side. Axial area very narrow, central area small, variable in shape formed by irregular interposition of shorter striae. Transapical striae strongly radiate in the middle and parallel close to the apices, 10-11 in 10 µm. Lineolae difficult to discern with LM. 35 in 10 µm.

Etymology. This taxon is dedicated to our friend and colleague Roland Schmidt, phycologist and ecologist in Mondsee/Salzburg, Austria.

Distribution and ecology. The taxon is only known (in high numbers) from the type locality. It seems to be confined to brackish and marine habitats but has been overlooked or confused with other taxa in the past.

This species is very similar to the complex of taxa around *N. salinarum*, and less similar when compared with *N. witkowskii* (Table 2).

***Navicula vaneei* Lange-Bertalot sp. nov. Figs 28-32.**

Type: Praep. E-Lok 82 in Coll. Lange-Bertalot, Botanical Institute J. W. Goethe University, Frankfurt am Main.
Type locality: River Reckerta near "Heiliges Meer" in northwestern Germany (*leg.* Ingeborg Krause, August 1993)

Latin diagnosis. *Valvae lanceolatae apicibus plerumque non protractis raro minime protractis, ad extremum obtuse rotundatis, 40-80 µm longae, 11-13 µm latae. Fissurae raphis modice laterales poris centralibus crasse signatis. Area axialis modice anguste linearis, area centralis distincte asymmetrica apparens, transverse rectangulata ad transverse ellipticam. Striae transapicales radiantes, denique convergentes, 8-10 in 10 µm. Lineolae valde crassae apparentes, 20-24 in 10 µm. SEM: Pori centrales ampli sed paulo hamati.*

Diagnosis. Valves lanceolate with ends which are, regularly, not protracted or, exceptionally, indistinctly protracted, apices obtusely rounded, 40-80 µm long, 11-13 µm broad. Raphe fissures moderately lateral with coarsely marked central pores. Axial area linear and moderately narrow, central area distinctly asymmetric, transversely rectangular to elliptic. Transapical striae radiate, convergent at the apices, 8-10 in 10 µm. Lineolae comparatively coarse, 20-24 in 10 µm. SEM: The large central pores with short, hook-like processes (Figs 35-36) are similar to *N. peregrina*.

Etymology. This taxon is dedicated to our friend and colleague Gert van Ee, phycologist and ecologist in Haarlem, Holland. He was the first to ask for the real identity of this taxon among the complex of several similar species around *Navicula rhynchocephala*.

Distribution. Until recently only known from several localities in Europe.

Ecology. It occurs infrequently, but locally abundantly in eutrophic inland waters, or freshwaters near sea coasts with moderately high conductivity.

This species was probably observed by other authors also in the past but not discerned from similar taxa, such as: *N. rhynchotella*, *N. slesvicensis*, *N. peregrina* and *N. meniscus*. For example it was not distinguished from *N. rhynchocephala* by Krammer & Lange-Bertalot (1986, fig. 31: 1-2). Germain (1981, fig. 69: 4-6) identified it as *N. rhynchocephala* var. *elongata* A. Mayer. *N. pseudolanceolata* Lange-Bertalot is (less) similar, but, has a distinctly different autecology, occurring, in oligotrophic slightly acid waters with very low conductivity.

Navicula witkowskii Lange-Bertalot, Iserentant & Metzeltin sp. nov. Figs 7-11.
Type: Praep. ■ 5894(1) in Coll. Brockmann, Natur-Museum Senckenberg, Frankfurt am Main
Type locality: Mouth of the River Weser, Bremerhaven (6.4.1934, *leg.* (?) C. Brockmann)

Latin Diagnosis. *Valvae lanceolatae vel elliptico-lanceolatae apicibus protractis rostratiformibus ad extremum obtuse rotundatis. Longitudo, 20-45 µm, latitudo, 9-12 µm. Fissurae raphis modice sive distincte laterales poris centralibus fere distantes inter se. Area axialis modice anguste linearis, area centralis variabilis quoad amplitudinem, circularis ad ellipti-*

cam. Striae transapicales valde radiantes ad apices versus parallelae denique convergentes, 10-12 in 10 µm. Lineolae fere difficiliter discernandae in microscopo photonico, 33 in 10 µm.

Diagnosis. Valves lanceolate or elliptical-lanceolate with protracted rostrate ends and obtusely rounded apices, 20-45 µm long, 9-12 µm broad. Raphe fissures moderately to more distinctly lateral with marked central pores in a somewhat distant position to each other. Axial area moderately narrow, linear, central area variable in extension, circular to elliptical. Transapical striae strongly radiate but parallel and finally convergent near the apices, 10-12 in 10 µm. Lineolae not easy to discern with LM, though not more than about 33 in 10 µm.

Etymology. This taxon is dedicated to our friend, colleague and co-worker, A. Witkowski from the University of Szczecin, Institute of Marine Sciences.

Distribution and ecology. It occurs frequently in brackish water of European coasts and river estuaries, whereas rarely in freshwaters with moderate to higher conductivity.

Up to now this taxon was either completely neglected and/or probably confused with *N. salinarum* or *N. digitoradiata* (Gregory) Ralfs (var. *rostrata* Hustedt), but agrees with *N. digitoradiata sensu* Brockmann (1950, fig. 2: 3). It occurs often with *N. salinarum* and *N. digitoradiata*, but is easy to distinguish in such associations (Table 2).

Table 2. Morphometric characteristic of newly described and related *Navicula* taxa.

Species	Length in µm	Width in µm	Striae in 10 µm	Lineolae in 10 µm	Shape	Apices	Central area
N. tripunctata (O. F. Müller) Bory	30-70	6-10	10-11	32	linear-lanceolate	obtusely rounded	rectangular
N. bourellyivera Lange-Bertalot, Witkowski & Stachura	30-55	10-12	9-11	20-21	lanceolate/ linear-lanc.	Acutely rounded	rectangular/ lanceolate
N. hanseatica Lange-Bertalot & Stachura	30-72	12-15	8-9	20-21	lanceolate	protracted acutely rounded	transversely expanded
N. rolandii Wunsam, Witkowski & Lange-Bertalot	30-47	10-11	10-11	35	linear-elliptical	cuneate obtusely rounded	small irregular
N. vaneei Lange-Bertalot	40-80	11-13	8-10	20-24	lanceolate	obtusely rounded	rectangular/ elliptical asymmetric
N. witkowskii Lange-Bertalot, Iserentant & Metzeltin	20-45	9-12	10-12	33	lanceolate/ elliptic-lanceolate	protracted rostrate	variable circular/ elliptical
N. digitoradiata (Gregory) Ralfs	25-80	7-28	7-14	32	lanceolate/ linear – lanceolate	obtusely rounded lanceolate	small circular
N. kefvingensis (Ehr.) Kützing	44-90	10-18	7-8.5	25-27	lanceolate	obtusely rounded	rectangular
N. meniscus Schumann	35-70	13-20	7-8	22	lanceolate	acutely rounded	rectangular expanded
N. peregrina (Ehr.) Kützing	40-180	10-30	5-61	8-20	lanceolate	obtusely rounded	rectangular/ elliptical

N. rhynchocephala Kützing	35-80	9-14	7-12	20-25	lanceolate	obtusely rounded	rectangular/ elliptical
N. rhynchotella Lange-Bertalot	35-60	10-16	8-11	20-25	lanceolate	protracted slightly capitate	transversely expanded
N. salinnarum Grunow	20-40	8-12	13-17	40	lanceolate	protracted slightly capitate	circular
N. slesvicensis Grunow	25-60	8-11	8-9	25	lanceolate	obtusely rounded	rectangular

The *Navicula cancellata* Donkin group (Figs 39-53)

Description: Valve faces and mantles not distinctly separated, but continuously strongly arched. Valve outlines linear-elliptical with broadly rounded apices. Raphe straight, central external endings deflected to the same side, close to each other. Internal raphe fissures in central position without raised siliceous sternum (Figs 58-59, 72). Terminal raphe endings strongly to moderately hooked to the same side, unlike in *Navicula sensu stricto* not in a polar but in a subpolar position (Fig. 45). Axial area narrow, central area relatively large. Transapical striae bold, composed of fine, regularly spaced lineolae. Girdle broad, composed of heteromorphic open bands.

This is a group of marine taxa with heavily silicified valves and broadly rectangular frustules in girdle view (Figs 41-43, 48-50, 52-53). Besides *N. cancellata*, *N. apiculata* Brébisson, *N. bipustulata* A. Mann, *N. crucifera* Grunow, *N. inflexa* Gregory, *N. mediterranea* Cleve & Brun, *N. northumbrica* Donkin, *N. retusa* Donkin, several until now undescribed taxa also belong in this group. Due to their comparatively broad girdle, taxa belonging to this group are rarely observed in valve view.

The *Navicula distans* W. Smith group (Figs 60-66)

Description: Frustules broadly rectangular in girdle view, valve faces slightly curved along apical axis. Valve outlines linear lanceolate with acutely rounded apices. Valve face (in SEM) flat in the middle, becoming arched towards apices. Raphe straight, external central raphe endings distinctly expanded. Terminal raphe endings curved to the same side. They continue up to the edge of the apex (Fig. 73). Internal raphe fissures distinctly deflected to one side of the raised raphe sternum terminating in a small helictoglossa. The central nodule is stauros-like, a shortened transapical bar (Fig 76). Axial area lanceolate, central area variable, mostly large, rectangular. The striae are composed of apically elongated, densely spaced slit-like foramina outside (Figs 60-62, 64-66). Internally they are positioned in comparatively very deep depressions (Fig. 74). Areolae on the interior seem to be much shorter than their external foramina (Figs 73-76). No internal occlusions with hymenes have been observed so far. Girdle composed of few (4) closed bands. The broad valvocopula has pectinate siliceous outgrowths (Fig. 63).

Species in this small group possess heavily silicified frustules, and include *N. distans* W. Smith and varieties, *N. pennata* A. Schmidt, probably *N. fortis* (Gregory) Ralfs, *N. longa* (Gregory) Ralfs and varieties and *N. spuria* Cleve. These are marine taxa with large and

robust cells, with the exception of *N. distans* var. *borealis* Grunow, the taxa are rather rarely reported.

The taxa included in this group occur in littoral marine environments. *N. distans* var. *borealis* occurs abundantly along European coasts up to the arctic Bear Island. *N. spuria* is reported predominantly from the warmer coasts e.g. the Mediterranean and Africa (Foged, 1975; Giffen, 1971). *N. fortis*, *N. longa* and *N. pennata* have been reported from European and American coasts of the Atlantic Ocean (A. Schmidt 1874, A. Schmidt *et al.*, 1874-1959; Peragallo & Peragallo, 1897-1908; Gemeinhardt, 1935; Hustedt, 1955).

The *Navicula platyventris* Meister group (Figs 15-22)

Description: Valve surface as observed in SEM is flat. In *N. platyventris* occurs a shallow depression along the raphe sternum. Raphe straight, external central raphe endings distinct, point-like. Internal central raphe endings continuous (Figs 78, 80). Terminal raphe endings strongly curved to the same side. Internal raphe fissures in central position. Unlike *Navicula sensu stricto*, the raphe sternum does not form a raised rib. Transapical striae are composed of S-shaped, sometimes irregular lineolae (Figs 77, 79). The areolae at the valve interior are covered with thin hymenes. The girdle is composed of four open bands. The valvocopula possesses short siliceous outgrowths (Fig. 78).

This is a small group of taxa which includes *N. platyventris* Meister, and *N. tropicoidea* Witkowski, Metzeltin & Lange-Bertalot. SEM examination will reveal whether or not *N. tropica* Meister, *N. raphoneis* Cleve and *N. perrhombus* Hustedt (Figs 94-99) belong to this group.

N. platyventris is predominantly distributed in warm regions of all oceans (e.g. Meister, 1935; Foged, 1975, 1987; Bafana & Witkowski, 1995), whereas *N. tropicoidea* has only been recorded from the type locality, namely Bear Island (Metzeltin & Witkowski, 1996).

The *Navicula starmachioides* Witkowski & Lange-Bertalot group (Figs 82-85)

Description: Valve faces arched along apical axis. Unlike *Navicula sensu stricto* there is no abrupt transition from valve face to mantle, but a gradual transition is observed over a steep slope. Raphe straight, exactly central in the middle of the valve, but slightly displaced to the valve margin near the apices. Internal raphe fissure deflected to one side of the raised raphe rib. This is similar to the conditions in *Navicula sensu stricto.* External central raphe endings simple, dot-like, terminal raphe endings simple, forming short slits or terminal pores indistinctly deflected to the same side. Transapical striae composed of slit-like foramina, areolae internally occluded by thin hymenes. At the apices a variable number of circumpolar areolae occur. The girdle is composed of four open, relatively broad bands (Figs 86-87).

This is a group of marine and brackish-water taxa, which are easily confused with those belonging to the group around *Navicula cancellata* and the newly established genus *Hippodonta* Lange-Bertalot, Metzeltin & Witkowski.

The *Navicula wasmundii* Witkowski, Metzeltin & Lange-Bertalot group
(Figs 100-110)

Description: Frustules rectangular in girdle view. Valves lanceolate to linear-lanceolate, with acutely to broadly rounded apices. Valve face flat without distinct change to the valve mantle. Raphe straight with simple, dot-like external central endings. Terminal raphe endings strongly hooked to the same side. Internal raphe fissures straight, central, within the raphe sternum which does not form a raised rib. Axial area very narrow, linear, central area small, rectangular. Transapical striae composed of simple lineolae. Areolae open externally as apically oriented slits and are internally covered by thin hymenes. Striae are regularly composed of only a few (2-3) lineolae. In each stria one or two lineolae occur along the valve margin and only one along the raphe. Lack of lineolae in the middle part of the valve gives an impression as if the taxa under consideration possess lateral areas (Figs 111-116). Our observations indicate, however, that the structure is different from lateral area recorded in *Fallacia* Stickle & Mann, *Lyrella* Karayeva (cf. Round *et al.*, 1990) or *Fogedia* (Witkowski *et al.*, 1997). On the other hand, observations on broken specimens indicate that the valve face is composed of two siliceous layers (Metzeltin & Witkowski, 1996).
This group is composed of only — so far as is known — a few small celled marine taxa which have often been confused with the complex around *Navicula perminuta* Grunow.

Other questionable groups in *Navicula sensu stricto*

Navicula pseudopima Hustedt and its forma *lanceolata* Hustedt (cf. Simonsen 1987, fig. 599:1-3) are without doubt members of the Lineolatae, but, are they really members of *Navicula sensu stricto*? Round *et al.* illustrate within *Navicula* (1990, p. 567, fig. j, k) a taxon which seems to belong to the group in question. Except an extremely convex valve shape with strong mantles, they possess a peculiar conopeum, but lack the typical hooked terminal raphe fissures. These features are unknown in the 150-200 species of *Navicula* which conform with the type species *N. tripunctata*. These typical *Navicula* taxa are characteristic elements of freshwater and brackish waters. However, the "atypical" groups under consideration here are elements of brackish to fully marine habitats, and not freshwaters.

Other examples of Naviculae Lineolatae which may not belong to *Navicula sensu stricto* are *Navicula gracilis* var. *recurva* Meister (Figs 67-68), and *N. flagellifera* Hustedt (Figs 33-34, 69-70). We compared our LM photos of *N. gracilis* var. *recurva* with SEM micrographs (phot. W. Güttinger) and found a channel-like to keel-like construction above the valve sternum. This feature is completely unknown in freshwater *Navicula* taxa. At first glance *N. flagellifera* possesses the typical valve features of *Navicula sensu stricto* When observed under SEM it reveals a peculiar construction of apices and internal raphe fissures somewhat similiar to *N. distans* and to *Trachyneis* Cleve (Figs 69-70). These and other examples will be published elsewhere.

DISCUSSION

SEM examination of the morphology of *Navicula* spp. even adopting the restrictive generic delimitation of Round *et al.* (1990) has revealed that, contrary to the recently expressed conviction (Round, 1996), at least six discrete groups can be distinguished. Our studies indicate that strict application of the generic diagnosis has the consequence of excluding from *Navicula* many taxa of the "Lineolatae". Only one of the groups (Table 3) under discussion here may be considered a subgenus in *Navicula sensu stricto*, *i.e.* the *N. starmachioides* group.

The designation of *N. tripunctata* as type of *Navicula* by Patrick (1959), emended by Cox (1979) has brought about important consequences for the circumscription of the genus. Subsequently Round *et al.* (1990) have emended the traditional diagnosis (Krammer & Lange Bertalot, 1986) of the Naviculaceae. Among diatomists there exists a distinct tendency to reject genera which do not represent "homogenous groups" of taxa. Following this strict concept, only the *N. starmachioides* group can be included in *Navicula sensu stricto* as an infrageneric rank, e.g. as a subgenus. The only important differences between *N. starmachioides* and *N. tripunctata* are the apical raphe endings and the heteromorphic girdle elements. All the other groups distinguished here (Table 3) show more significant morphological differences from the diagnosis of *Navicula*. In particular this is seen in the following features: position of the internal raphe fissures, structure of the striae, and the girdle.

In general the internal raphe fissures of *Navicula sensu stricto* open laterally and are positioned within a thin, raised siliceous rib on the sternum. In *N. platyventris* and in *N. wasmundii* the internal raphe fissures have a central position continuously and resemble recently delimited genera, *i.e. Fogedia* (Witkowski *et al.*, 1997) and *Hippodonta* (Lange-Bertalot *et al.*, 1996). However, they differ from *Fogedia* and *Hippodonta* by having strongly curved apical raphe endings, which are typical for *Navicula*.

In the *N. distans*, group the raphe internal structure resembles *Navicula sensu stricto* but there is a distinct difference. In addition the structure is reminiscent of *Trachyneis*, especially at the apices and in the middle (e.g. Round *et al.*, 1990; Medlin, 1991). Two features provide the most pronounced evidence in a support of this: a distinct stauroid silica accumulation at the central nodule and the internal raphe central ends. The transapically expanded central nodule can also be observed with LM. The terminal raphe ends of a group around *N. distans* also very much resemble those in *Trachyneis* and in *Rhoiconeis* Grunow (Round *et al.*, 1990; Medlin, 1991). However, there are also important differences between a group around *N. distans* and *Trachyneis* the most important of which is the stria structure.

The most prominent feature of *Navicula* apart from the boat-like outline in light microscope, is the structure of the striae, which are composed of apically elongated slits, the so-called lineolae (e.g. Cox, 1979, 1987b; Cox & Ross, 1981; Krammer & Lange-Bertalot, 1986; Round *et al.*, 1990; Round, 1996). The taxa we have studied with respect to this feature show some variability. In *Navicula tripunctata* and numerous related taxa lineolae are slit-like foramina. The most similar structure is exhibited by the *N. starmachioides* and *N. wasmundii* groups. *N. platyventris* shows a modification in form of S-curved foramina. The striae of species in the *N. distans* group have a different fine structure: externally the areolae open as very thin, densely spaced slits, while internally

they lie in deep depressions between two raised transapical costae (virgae). The internal width of the striae (depressions = alveoli) seem to be smaller than that of the external foramina. This feature is possibly observed even at different focus planes with LM. At upper focus, the striae are distinctly broader than at lower focus.

The girdle structure is also variable. *Navicula sensu stricto* and the *N. distans* and *N. platyventris* groups have the same principle type of girdle construction. Copulae are open, plain bands, while valvocopulae have pectinate outgrowths. Their length, however, is different and varies from very short or even missing in *Navicula sensu stricto* (see Cox, 1987b, 1995) through intermediate in *N. platyventris* to long outgrowths in *N. distans*. The girdles of *N. cancellata*, *N. starmachioides* and *N. wasmundii* possess similar stuctures and are composed of two different types of bands. Medlin (1991) uses the term heteromorphic girdle in such cases. She observed such girdles in *Rhoiconeis* and *Trachyneis*.

Interesting is a comparison of the *N. starmachioides* group with the newly established genus *Hippodonta*. Superficially both taxa resemble each other, especially with respect to the apical raphe endings, girdle construction and the presence of a peculiar "horse teeth" arrangement of foramina at the apices. In the light of the generic diagnosis of *Navicula*, the *N. starmachioides* group may be included in *Navicula*, whereas *Hippodonta* does not fit the diagnosis. Their discrimination in LM may be difficult. The key to solve this problem may be their different salinity requirements. *Hippodonta* comprises predominantly fresh — to slightly brackish water species, whereas the *N. starmachioides* group contains more brackish water and fully marine forms.

CONCLUDING REMARKS

The results of our study show inevitably that, despite efforts taken to clear the taxonomic problems within *Navicula sensu stricto* the aim of recognising a homogenous group of taxa has not yet been attained. We were able to vindicate our suspicion that, even within *Navicula sensu stricto*, there are several groups of species, which in light of a restricted generic diagnosis cannot be placed in the genus *Navicula*. The taxa which, based on LM observations, apparently belong to *Navicula*, studied with the aid of SEM have to be excluded from this genus if we follow the generic diagnosis. In this paper we illustrate six subgroups within *Navicula sensu stricto*, only one of which, *i.e.* the *N. starmachioides* group, in our opinion may be recognized as a subgenus of *Navicula*. Since we anticipate that more subgroups will be found in the future, we do not propose taxonomic changes at the generic level. It might be more appropriate to emend the generic diagnosis than to create new small genera, but it would not be an easy task.

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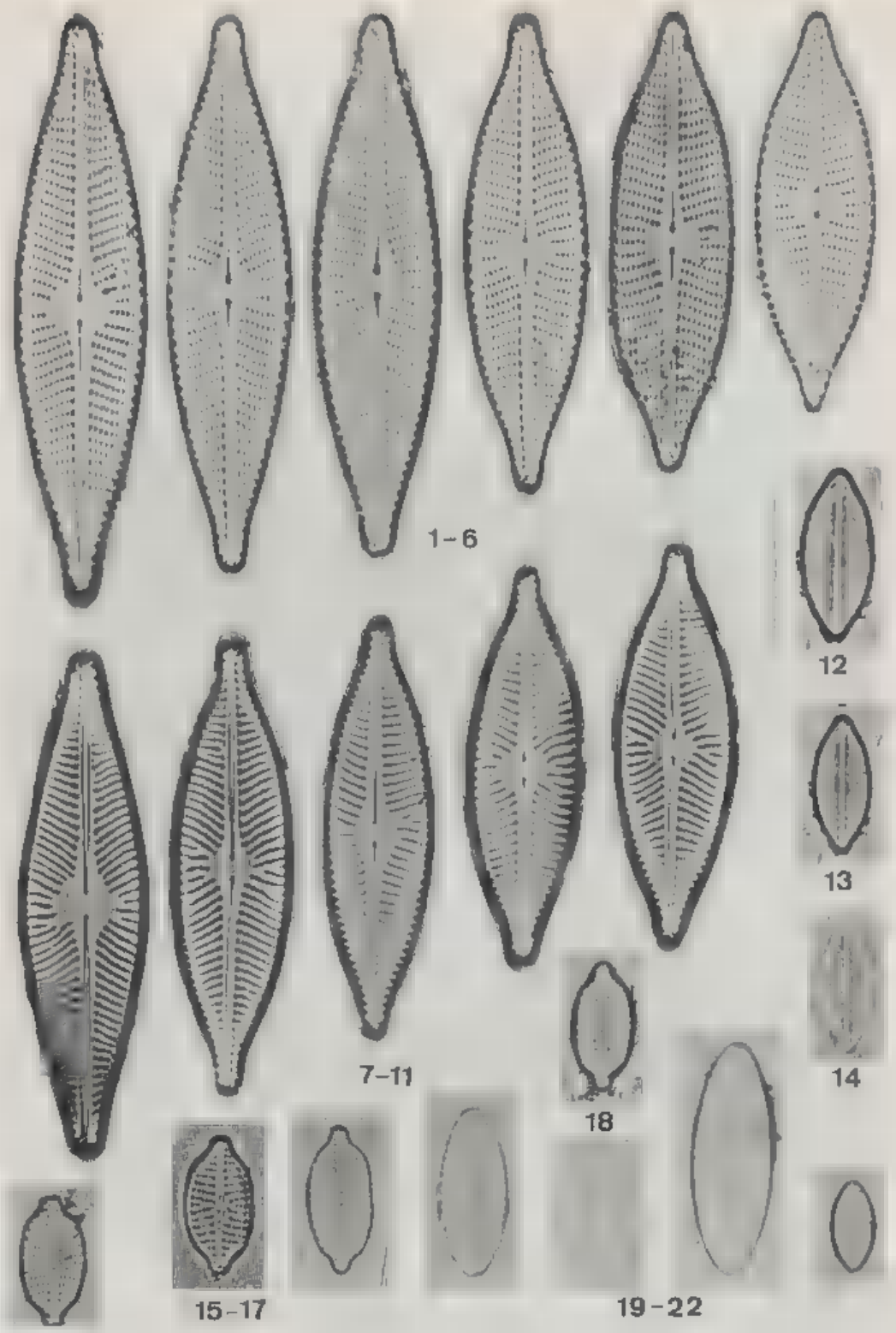
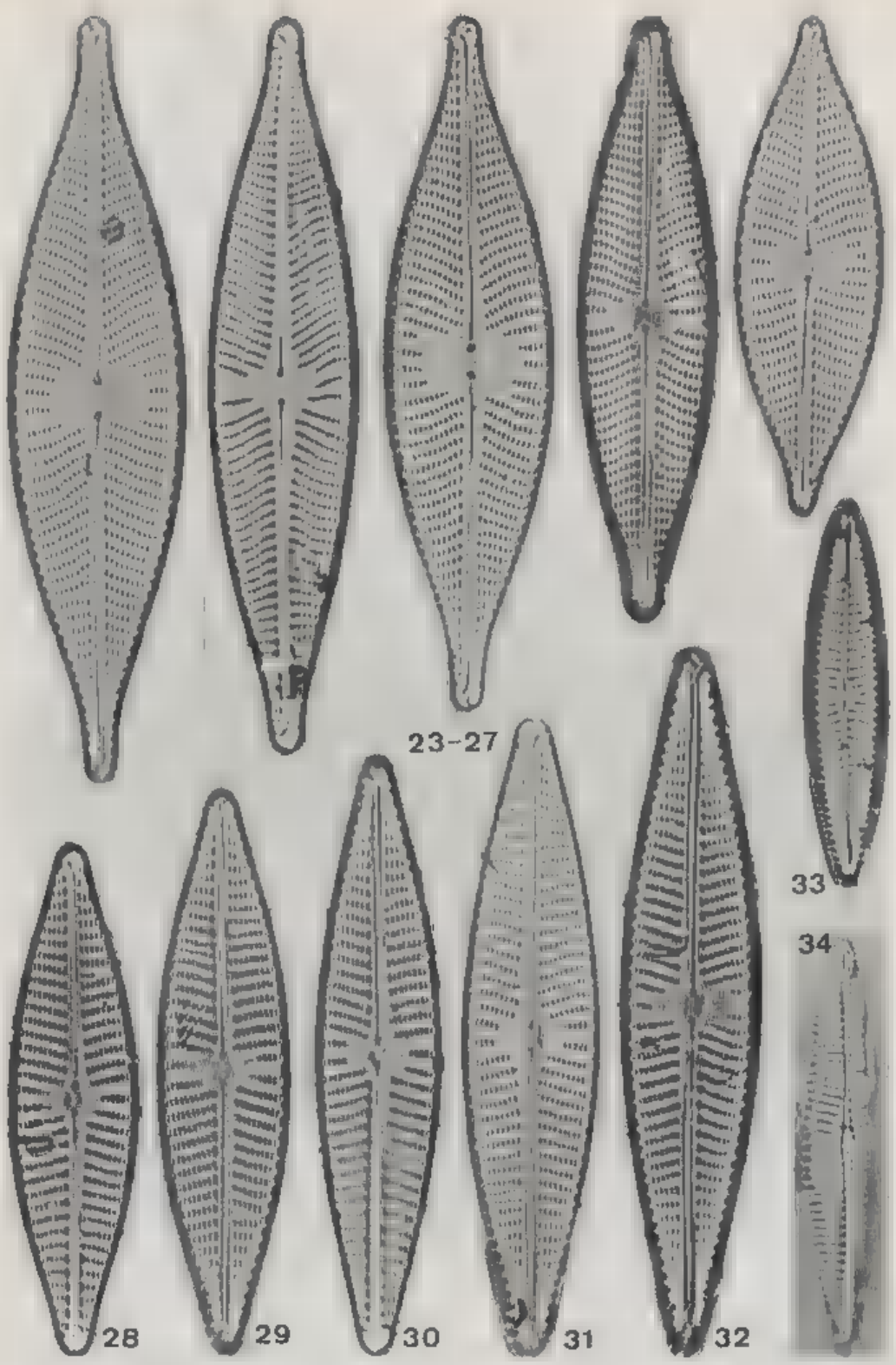
Table 3. The characteristic features of groups distinguished within *Navicula sensu stricto*

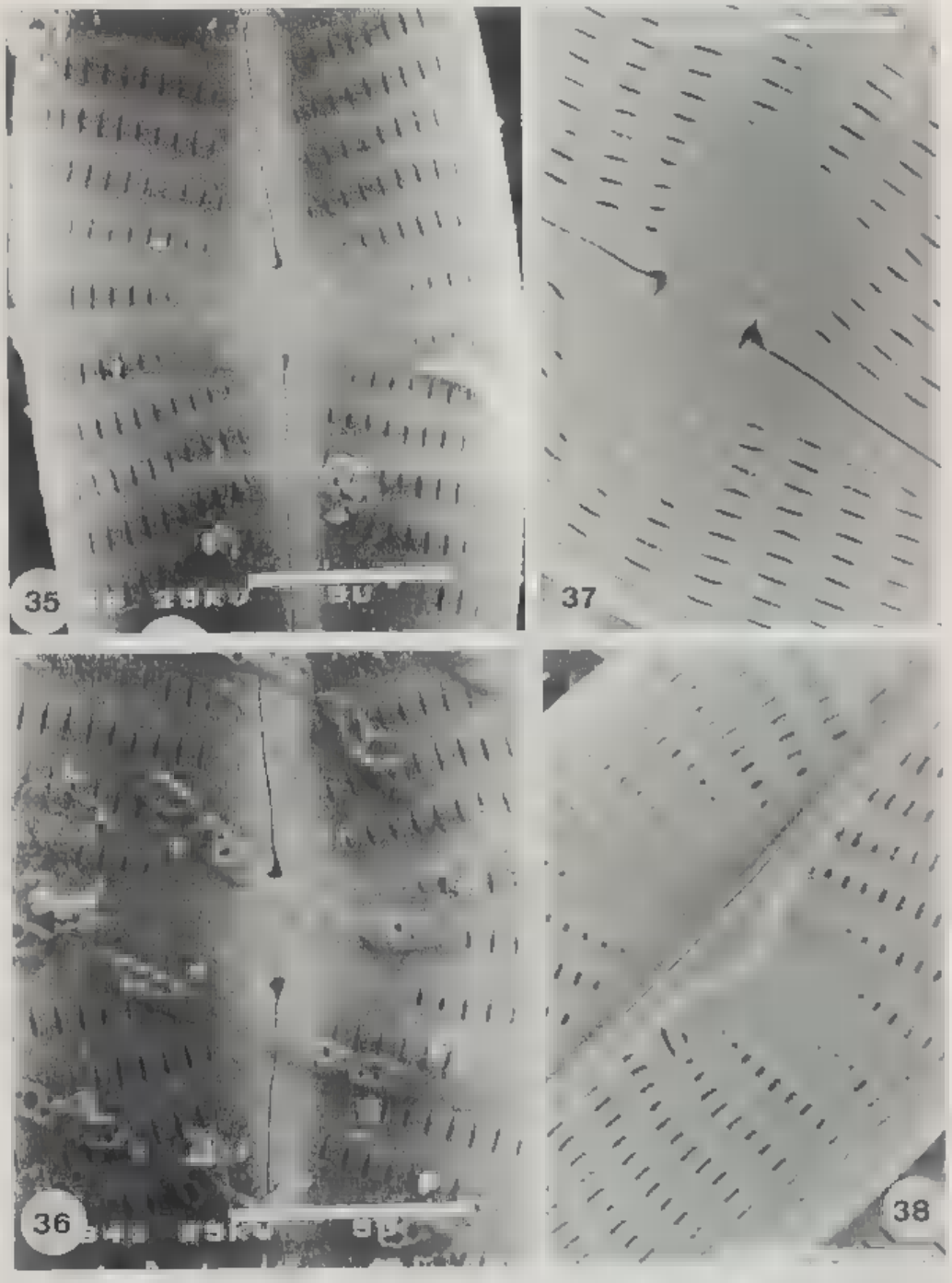
Taxonomic group	Shape of lineolae	Striae	Central raphe endings	Terminal raphe endings	Raphe rib on sternum	Internal raphe fissure	Internal central raphe endings	Cingulum
Navicula Section Naviculatae	slit-like	numerous lineolae	simple, filiform	hooked in polar position	present	deflected	discontinuous	open bands
N. cancellata group	slit-like, (fine)	numerous lineolae	deflected	hooked in subpolar position	absent	central	discontinuous	open bands
N. distans group	slit-like, (fine)	numerous lineolae	expanded	deflected	present	deflected	discontinuous	broad, with pectinate outgrowths
N. platyventris group	S-shaped	numerous lineolae	simple, filiform	hooked	absent	central	continuous	open with outgrowths
N. starmachioides group	slit-like	numerous lineolae	expanded	simple or slightly deflected	present	deflected	discontinuous	broad, open bands
N. wasmundii group	slit-like	2-3 lineolae	simple, filiform	hooked or deflected	absent	central	discontinuous	narrow, open bands



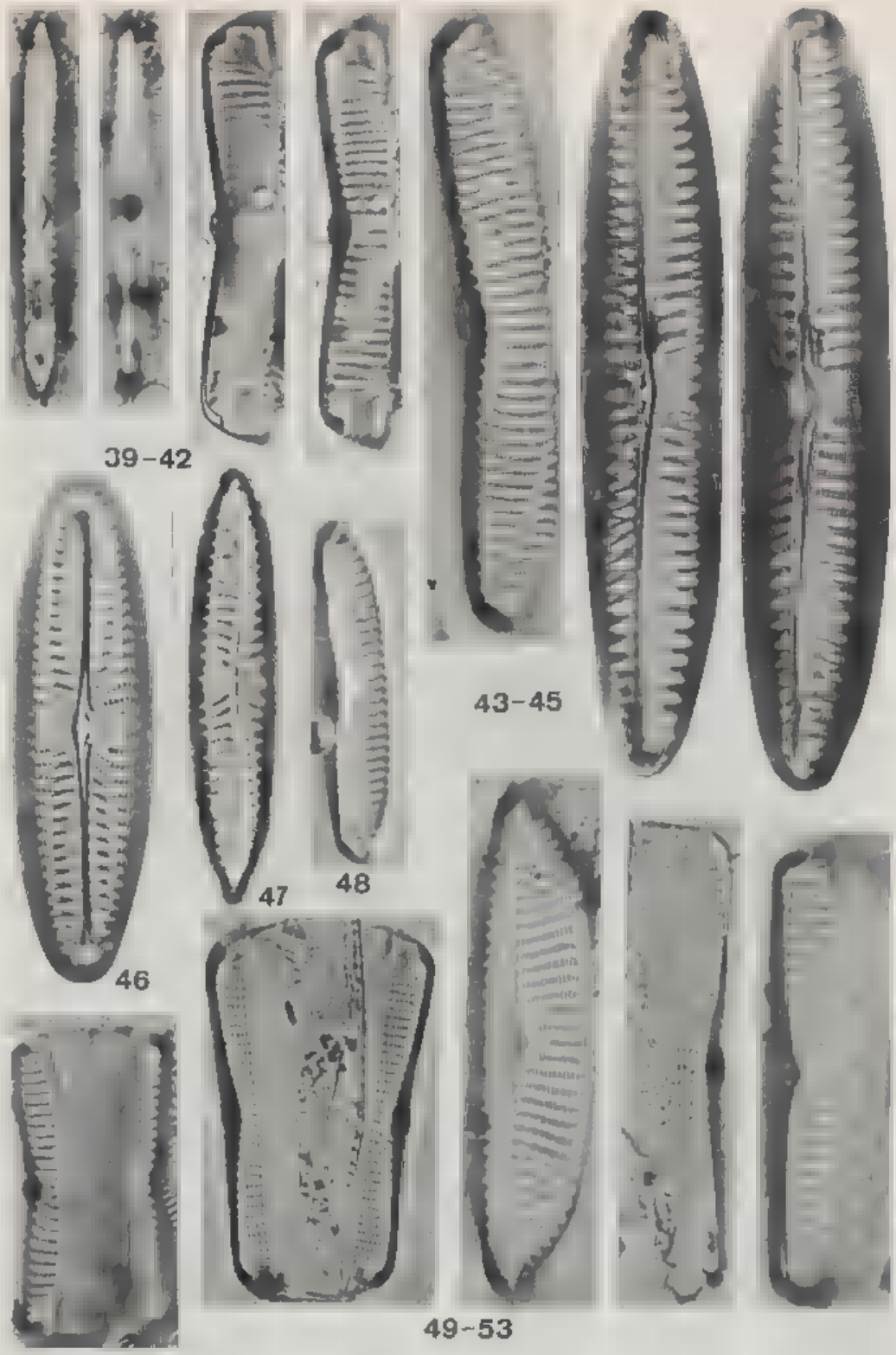
Figs 1-21. Figs 1-6. *Navicula bourrellyivera*, specimens from the salt marsh in Władysławowo, holotype slide. Figs 7-11. *Navicula witkowskii*, specimens from North Sea tidal flats, holotype slide. Figs 12-14. *Navicula viminoides*, specimens from the Mississippi Delta. Figs 12-13. The same valve at different magnification. Figs 15-18. *Navicula platyventris*. Fig. 15. Specimen from Fiji Islands. Fig. 16. Specimen from the Gulf of Oman. Fig. 17. Specimen from Kenya. Fig. 18. Specimen from South Africa. Figs 19-22. *Navicula tropicoidea*, specimens from Bear Island. Scale bar in Fig. 1 = 10 µm for Figs 1-11; 13-21, scale bar in Fig. 12 = 10 µm for Figs 12, 22.



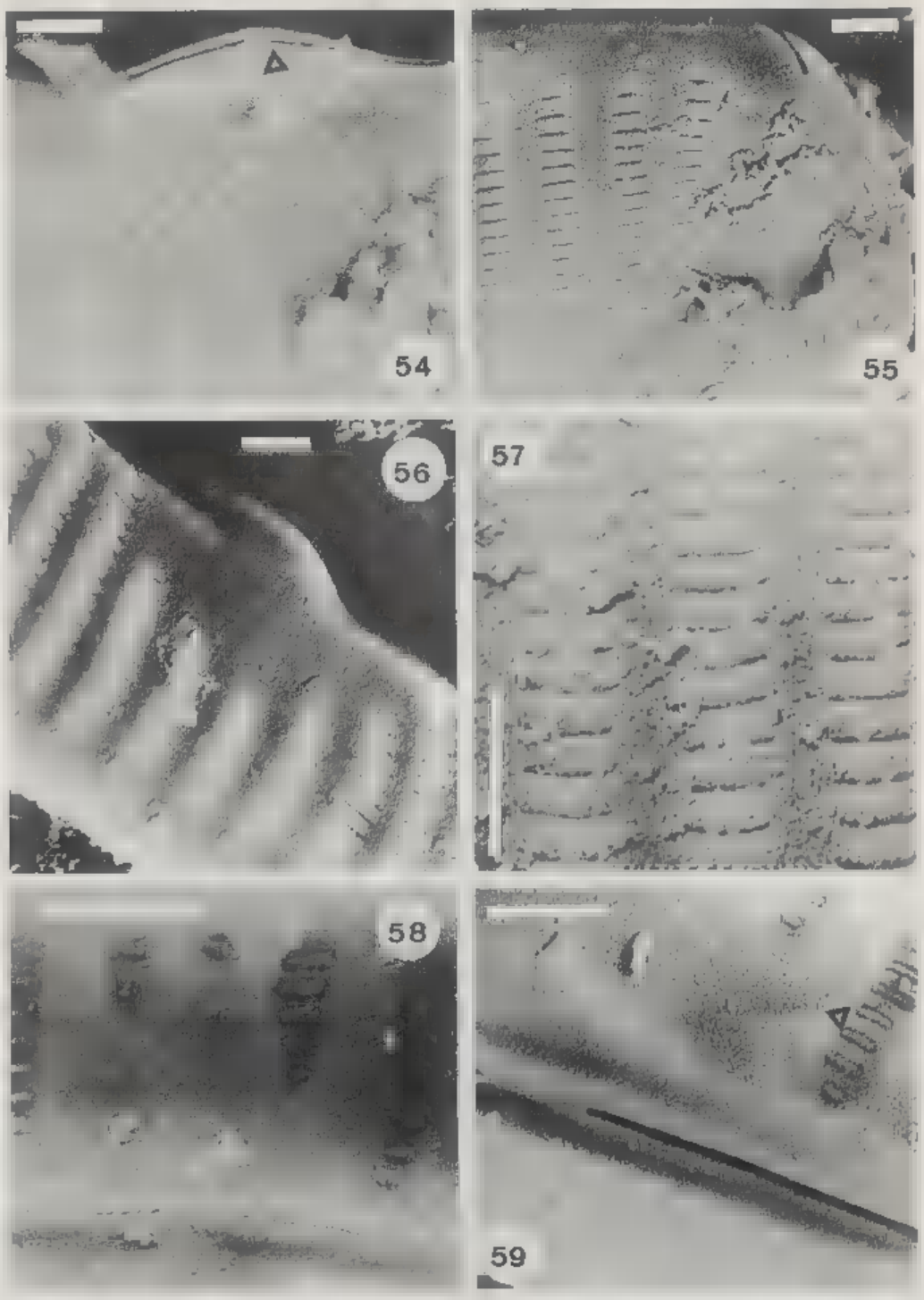
Figs 23-34. Figs 23-27. *Navicula hanseatica*, specimens from salt marsh in Władysławowo, holotype slide. Figs 28-32. *Navicula vaneei*. Figs 28-30 and 32. Specimens from River Reckerta, northwestern Germany, holotype slide. Fig. 31. Specimen from River Weser, sample No 1052, coll. Hustedt. Figs 33-34. *Navicula flagellifera*, specimens from the Mississippi Delta. Scale bar = 10 µm.



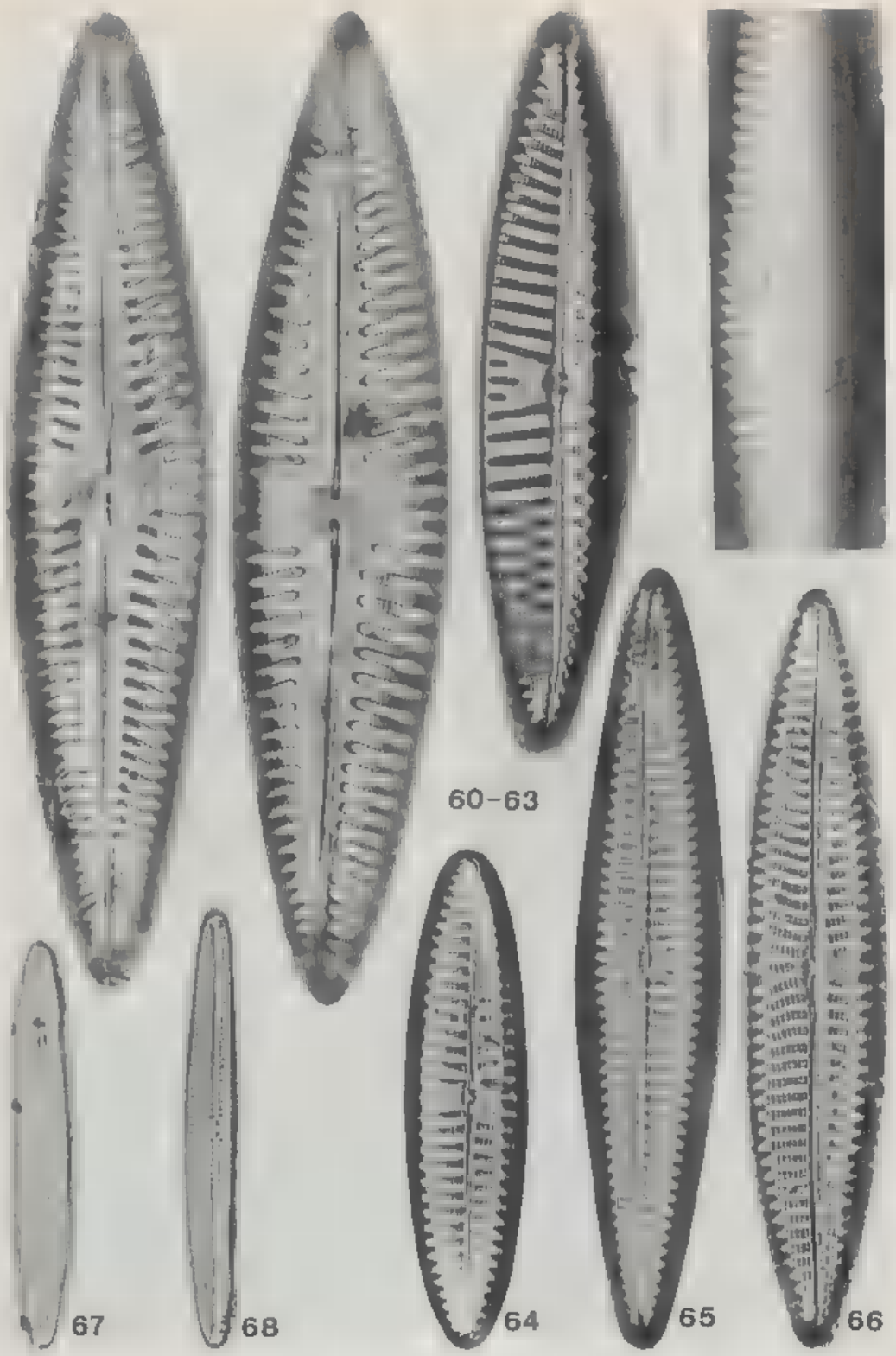
Figs 35-38. Figs 35-36 *Navicula vaneei*, SEM. Figs 35-36. Valve face, specimens from the type locality. Fig. 35. Middle part of the valve exterior showing central raphe pores and central area. Fig. 36. Central area of the specimen covered with regularly structured particles (siliceous skeletons of unknown organisms?). Figs 37-38. *Navicula hanseatica*. Specimens from the type locality. Fig. 37. Middle part of the valve exterior at higher magnification showing central raphe endings. Fig. 38. Valve interior typical of *Navicula sensu stricto*, characteristic position of the raphe sternum with raphe fissures. Scale bars = 5 µm.



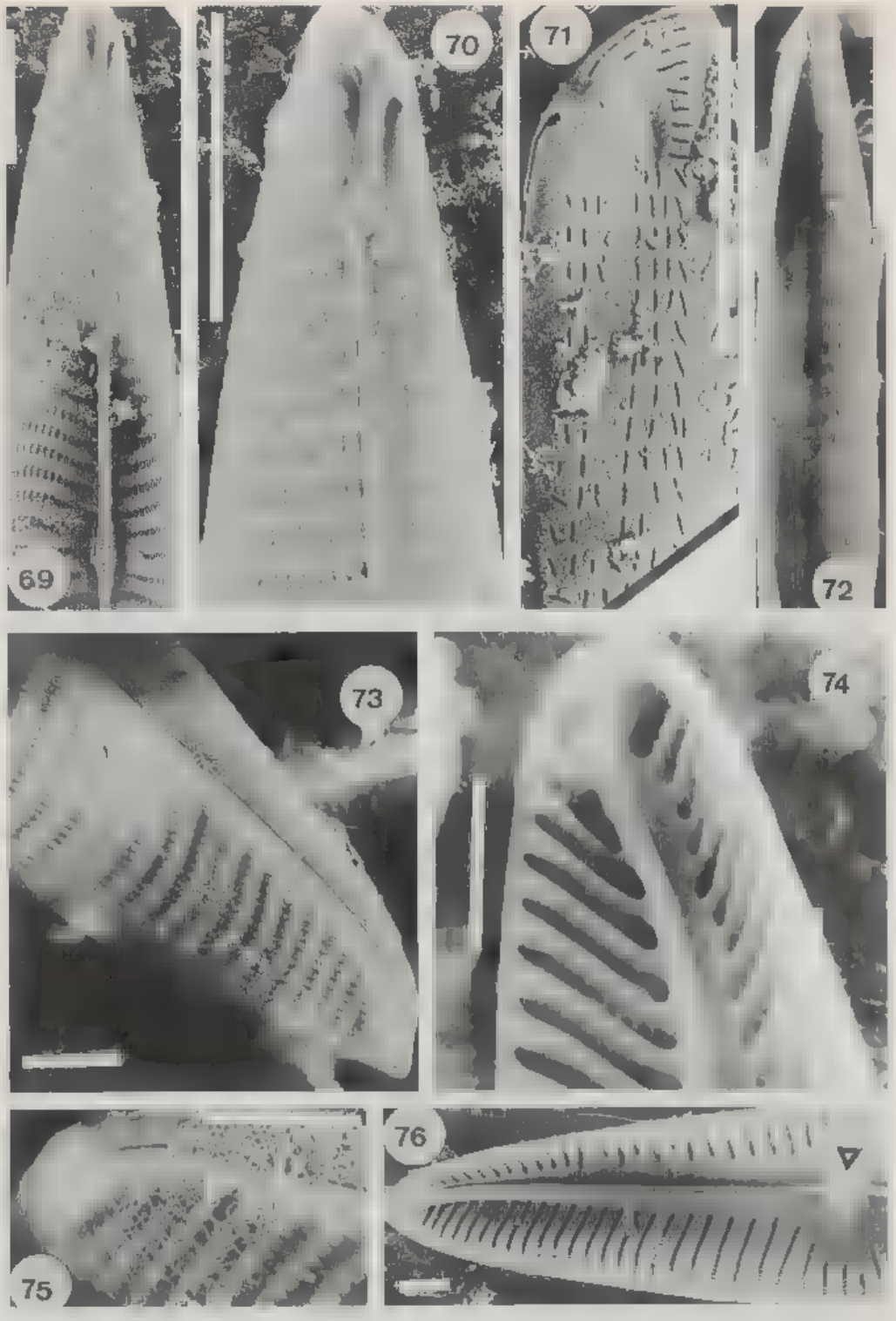
Figs 39-53. *Navicula cancellata* group. Figs 39-42. *Navicula* aff. *retusa*, specimens from the Mississipi Delta. Fig. 39-40. Valve face view of the same specimen at different foci. Fig. 39. Focus on the central nodule. Fig. 40. Focus at the raphe terminal endings (arrowhead). Figs 41-42. Girdle view of the same specimen at different foci showing the presence of an unusual central nodule (cf. SEM micrographs Figs 54, 56). Figs 43-46, 53. *Navicula cancellata*. Figs 43, 53. Specimens from Bear Island. Fig. 44-45. Specimens from the coast of Crete, Mediterranean Sea. Fig. 46. Specimen from Narvik. Figs 47-49. *Navicula bipustulata*. Fig. 47. Specimen from Narvik. Figs 48-49. Specimens from the Gulf of Gdańsk. Figs 50-51. *Navicula crucifera*. Fig. 50. Specimen from Drake's Bay, north of San Francisco, California. Fig. 51. Specimen from La Rochelle, Atlantic coast, France. Fig. 52. *Navicula northumbrica*, specimen from La Rochelle, Atlantic coast, France. Scale bar = 10 µm.



Figs 54-59. SEM. *Navicula cancellata* group. Figs 54-59. *Navicula* aff. *retusa*, SEM, specimens from the Mississippi Delta. Figs 54, 56. Middle part of the valve exterior. Fig. 54. External central raphe endings (arrowhead). Fig. 56. Central nodule in the girdle view. Fig. 55. External view of valve apex in girdle view. Fig. 57. External areolae openings at higher magnification. Figs 58-59. Interior of the valve centre showing centrally positioned raphe slits and the absence of a raised sternum. Note the presence of hymenate occlusions (arrowhead). Scale bars = 1 µm.

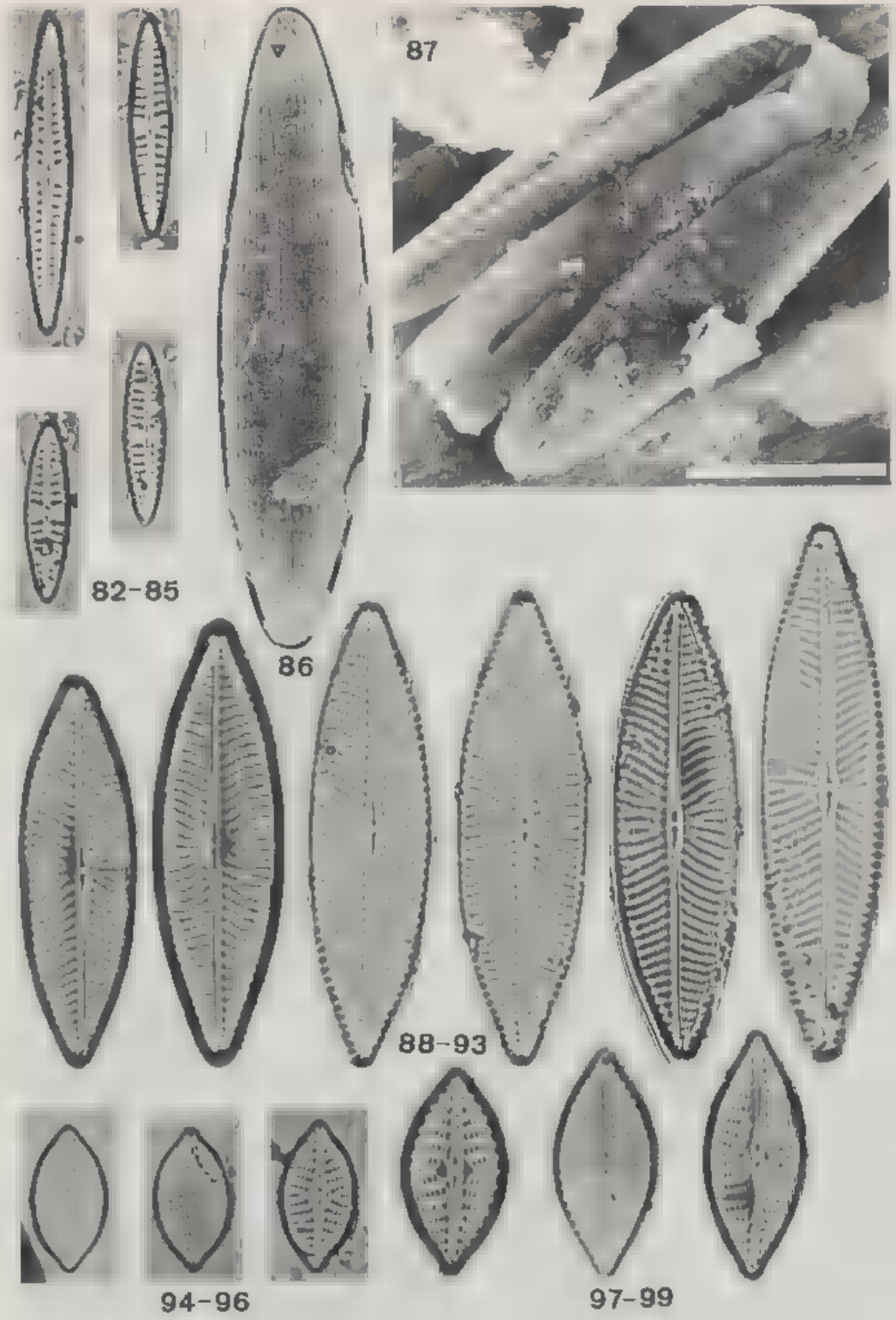


Figs 60-68. Figs 60-66. *Navicula distans* group. Figs 60-61, 63. *Navicula distans* var. *borealis*. Figs 60-61. The same specimen at different foci. Fig. 60. Focus on the valve interior. Fig. 61. Focus on the external valve surface. Note different appearance of the striae and of the central raphe pores. Fig. 63. Centre of the pectinate valvocopula of *N. distans* var. *borealis*. Figs 60-61, 63. Specimens from Bear Island. Fig. 62. *Navicula pennata*. Figs 64-65. *Navicula* aff. *spuria*. Figs 62, 64. Specimens from Drake's Bay, north of San Francisco, California. Fig. 65. Specimen from the Indian Ocean coast, Kenya. Fig. 66. *Navicula longa var. irregularis*, specimen from the Gulf of Campeche. Figs 67-68. *Navicula gracilis var. recurva*. Fig. 67. Specimen from the Azores, Atlantic Ocean. Fig. 68. Specimen from La Rochelle, Atlantic coast, France. Scale bar in Fig. 60 = 10 µm for Figs 60-61, 63; scale bar in Fig 62. = 10 µm for Figs 62, 64-68.

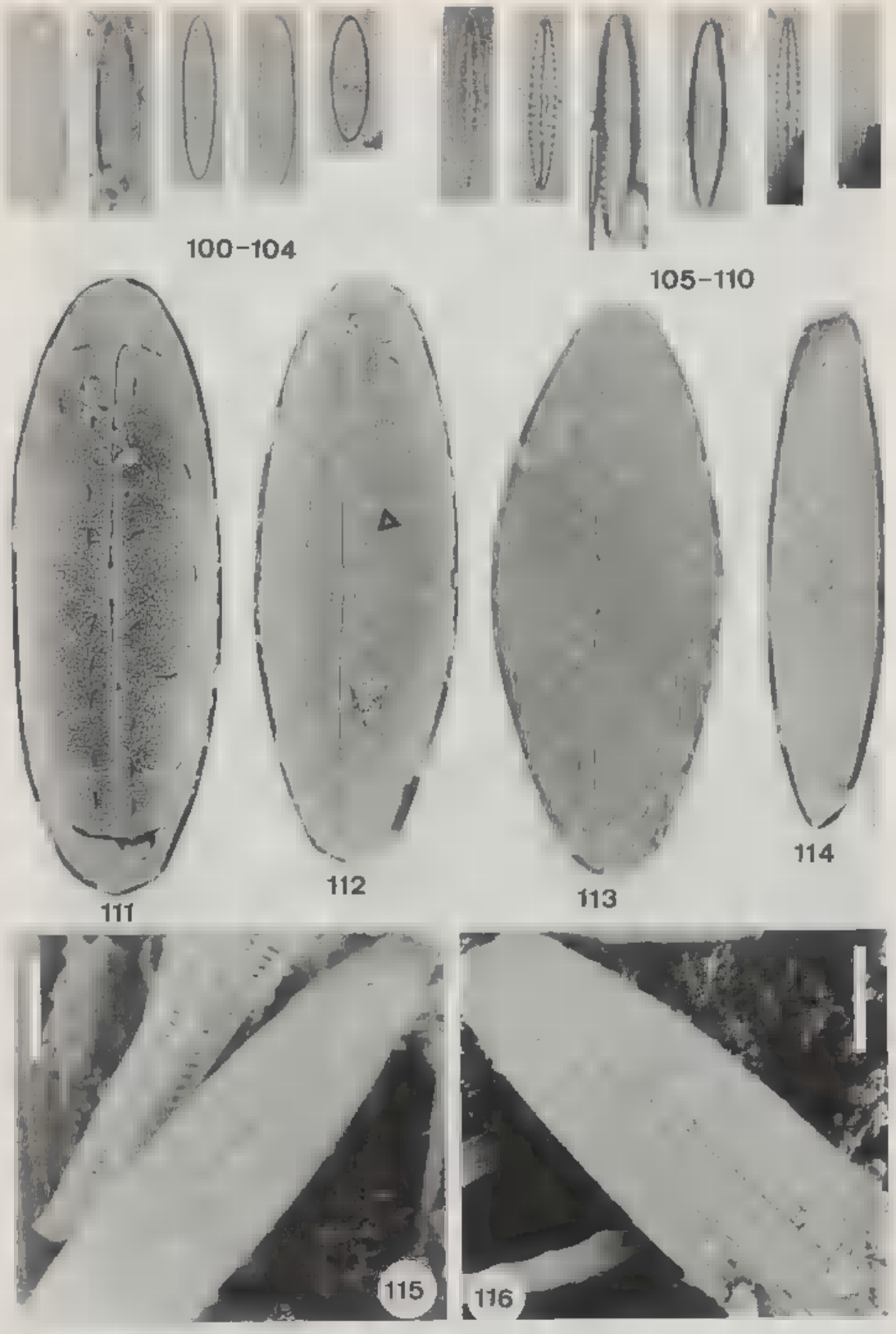


Figs 69-76. SEM. Figs 69-70. *Navicula flagellifera*. Fig. 69. Valve interior showing centrally positioned internal raphe slit which is located within a raised siliceous rib. Fig. 70. Apical part of the specimen illustrated in Fig. 69. showing the internal areola openings the interior of the valve apex. Fig. 71. *Navicula* aff. *retusa*. Valve exterior seen from the girdle side showing a simple, slightly deflected apical raphe ending. Fig. 72. Small *Navicula* sp. valve structure typical of the group around *N. cancellata*, note simple, central internal raphe slits. Figs 69-72. Specimens from the Mississipi Delta. Figs 73-76. *Navicula distans* var. *borealis*. Fig. 73, 75. Apical part of the valve exterior showing external raphe ending. Fig. 74. Apical part of the valve interior. Fig. 76. Valve interior, note the presence of distinctly thickened central nodule (arrowhead). Figs 73-76. Specimens from Bear Island. Scale bars = 5 µm.

Figs 77-81. SEM. *Navicula platyventris* group. Figs 77-78, 80. *Navicula platyventris*. Fig. 77. Valve exterior showing irregular external openings of the areolae. Fig. 78. Valve interior, note the presence of simple, centrally positioned internal raphe slit, absence of central nodule and the valvocopula with short outgrowths (arrowhead). Fig. 80. Valve interior with hymenate areola occlusions. Fig. 79. *Navicula* sp. Valve exterior with S-shaped external areola openings. Figs 77-80. Specimens from Kok-tao Island, Thailand. Fig. 81. *Navicula tropicoidea*, specimen from Bear Island. Valve interior showing simple centrally positioned raphe internal slits and valvocopula with short outgrowths. Scale bars = 1 μm.



Figs 82-99. Figs 82-87. *Navicula starmachioides* group. Figs 82-86. *Navicula starmachioides*, specimens from the Gulf of Gdańsk. Fig. 86. SEM. Valve external view, note the presence of slightly curved apical raphe endings (arrowhead). Fig. 87. Complete frustule of an unidentified *Navicula* sp. showing the valve exterior and interior. The valve interior shows presence of laterally deflected raphe internal slits. Specimen from the Littorina sediments of the Mecklenburg Bay, core No. 564024. Figs. 88-93. *Navicula rolandii* specimens from the type slide. Figs 94-96. *Navicula* aff. *perrhombus*. Figs 94-95. Specimens from Tonga, Indian Ocean, Tanzania. Fig. 96. Specimen from the coast of Crete, Mediterranean Sea. Figs 97-99. *Navicula perrhombus*. Fig. 97. Specimen from Fiji Islands, Coll. Foged. Fig. 98. Specimen from Eilat, the Red Sea. Fig. 99. Specimen from La Reunion, Indian Ocean. Scale bar in Fig. 83 = 10 µm for Figs 82-85 and 88-99, scale bars in Figs 86-87 = 5 µm.



Figs 100-116. *Navicula wasmundii* group. Figs. 100-104. *Navicula wasmundii*. Figs 100-101. Specimens from Bear Island. Figs 102-104. Specimens from the Kattegat. Figs 102-103. The same specimens photographed at different foci. Figs 105-110. *Navicula syvertsenii*. Figs 107-108. Specimens from Bear Island. Figs 105-106, 109-110. Specimens from Kattegat photographed at different foci. Figs 111-116. SEM. Fig. 111. Valve external view of *Navicula wasmundii* showing the stria structure, specimen from Bear Island. Figs 112-113. *Navicula* sp. Fig. 112. Valve internal view showing centrally positioned internal raphe slits and hymenate areola occlusions (arrowhead). Fig. 113. Valve exterior showing arrangement of the areolae external openings. Figs 112-113. Specimens from Kok-tao Island, Thailand. Figs 114-116. *Navicula syvertsenii*. Figs 114-115. External views at different magnifications, specimen from Bear Island. Fig. 116. External view of valve seen in girdle view, specimen from Franz Joseph Land. Scale bar in Fig. 100 = 10 µm for Figs 100-101, 107, scale bar in Fig. 102 = 10 µm for

DESMID ZYGOSPORES FROM FRENCH GUIANA AND THE PHENOMENON OF SIMULTANEOUS CONJUGATION IN MULTI-SPECIES ASSEMBLIES

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ABSTRACT — Sexual reproduction stages of fifteen desmid taxa were encountered in a tychoplankton sample from a shallow rain-forest pool in French Guiana. Zygospores are depicted for nine taxa. Those of *Cosmarium dimaziforme* (Grönbl.) A.M. Scott *et* Grönbl., *Euastrum ciastonii* var. *apertisinuatum* Scott *et* Prescott, *Groenbladia bourrellyi* stat. et nom. nov. (synonymous to *Hyalotheca neglecta* var. *major* Taylor), *Staurastrum eskolense* nom. nov. et stat. nov. (synonymous to *S. elegantissimum* var. *brasiliense* Förster) and *Xanthidium trilobum* Nordst. are new to science. The phenomenon of simultaneous conjugation by a number of different desmid taxa, also known from literature, might be explained by the production of non-species-specific pheromones inducing mating activity in a number of related algal species.

RÉSUMÉ — Les stades de reproduction sexuée de quinze desmidiées ont été observés dans un échantillon de tychoplancton ■ provenance d'une mare peu profonde de la forêt tropicale humide de Guyane française. Des zygospores ont été figurées chez neuf de ces taxons. Ceux de *Cosmarium dimaziforme* (Grönbl.) A.M. Scott *et* Grönbl., *Euastrum ciastonii* var. *apertisinuatum* Scott *et* Prescott, *Groenbladia bourrellyi* stat. et nom. nov. (synonyme de *Hyalotheca neglecta* var. *major* Taylor), *Staurastrum eskolense* nom. nov. et stat. nov. (synonyme de *S. elegantissimum* var. *brasiliense* Förster) et *Xanthidium trilobum* Nordst. sont nouveaux pour la science. Le phénomène de conjugaison simultanée d'un bon nombre de desmidiées, connu aussi dans la littérature, pourrait être expliqué par la production de phéromones non spécifiques au niveau de l'espèce, induisant l'activité d'accouplement chez un certain nombre d'algues apparentées. (Traduit par la Rédaction)

KEYWORDS: desmids, freshwater algae, zygospores, conjugation, pheromones, French Guiana

INTRODUCTION, MATERIAL AND METHODS

In February 1995, a number of freshwater habitats in northeastern French Guiana were sampled and searched for desmids. One small, rather shallow pool with ochreous-coloured water, near the settlement of Eskol, just north of the road from Roura (*ca* 20 km south of Cayenne) appeared to be remarkably rich in desmid zygospores. The

pool was partly filled in with *Utricularia* (presumably *U. myriocista* St. Hil. *et* Girard), which served as the substrate for the algae sampled. To this end, *Utricularia* material was squeezed out. In the thus obtained samples also electric conductivity was determined, using a portable WTW-LF92. Conductivity of the sample containing the zygospores described below measured 51 $\mu S\ cm^{-1}$. Shortly after collecting, the samples were fixed with formaldehyde to a final concentration of about 4%. Algal material was studied light microscopically (Wild M20) and drawings were made with the aid of a drawing tube. The sample containing the below-described zygospores is admitted into the algal collection of the Amsterdam Herbarium, under no. 95.05.

OBSERVATIONS

Cosmarium dimaziforme (Grönbl.) A.M. Scott *et* Grönbl.
var. ***concavum*** Förster *ex* Förster (Figs 5-8)

Cosmarium dimaziforme, a species with a predominantly Latin American distribution (e.g. Förster, 1982; Thérézien, 1985), includes several, closely related varieties, all known only in the vegetative state. In the Eskol sample, zygospores of var. *concavum* (Förster, 1982) were encountered. The spores were globular and ornamented with rather short spines arising from a conical base (Figs 7-8). Zygospores, including spines were 23-35 µm in diameter.

Euastrum ciastonii Racib.
var. ***apertisinuatum*** Scott *et* Prescott (Figs 1-4)

Euastrum ciastonii, known from North and South America (Krieger, 1937), has only been recorded once in a sexual state (Prescott & Scott, 1945, t. 8: 9). The accompanying figure is rather schematic, showing a globular, spiny spore with a single semicell next to it. To date, var. *apertisinuatum*, differing from the nominal variety by a widely opened sinus, was only known in vegetative state. In the Eskol sample, zygospores 42-50 µm in diameter were encountered. The shape accords with that of the nominal variety.

Groenbladia neglecta (Racib.) Teiling (Figs 9-12)

In our Eskol sample, two species of *Groenbladia* were present. Specimens of the most common one, depicted in Fig. 9, are in good agreement with *G. neglecta* as originally described by Raciborski (1895). According to his diagnosis, *G. neglecta* is characterized by more or less barrel-shaped semicells, showing 6-8 transverse rows of marked cell wall pores. Zygospores of *G. neglecta* were recorded by West & West (1898, under the name of *Hyalotheca neglecta* Racib.) from England, by Scott & Grönblad (1957, concerning var. *elongata* Scott *et* Grönbl.) from the southeastern United States, and by Grönblad *et al.* (1968, also referring to var. *elongata*) from tropical West Africa. Whereas West & West (*loc. cit.*) described the zygospores of *G. neglecta* as rounded and smooth-walled, zygospores of its var. *elongata* were found to be quadrangular with slightly retuse to straight sides.

Zygospores (Figs 10-12) belonging to *G. neglecta* cells answering Raciborski's diagnosis were abundant in the Eskol sample. They were more or less globular and smooth-walled and resembled those figured by West & West (*loc. cit.*), their dimensions — *ca* 20 to 30 µm in diameter — are comparable.

Groenbladia bourrellyi stat. et nom. nov. (Figs 13-15)
Replaced synonym: *Hyalotheca neglecta* var. *major* Taylor 1935, p. 222, t. 49: 14.

The second species of *Groenbladia* differs from *G. neglecta* in a number of features. Semicells are cylindrical with their greatest width at the base rather than in the midst (so chimney-pot-shaped rather than barrel-shaped; Fig. 13). The cell wall is somewhat thicker than in *G. neglecta* and does not show the transverse rows of pores. Pores, if distinguishable at all, are not arranged in a particular pattern. Zygospores are angular (Figs 14-15) versus rounded in *G. neglecta*. Screening of literature shows that this taxon has been recorded under *G. neglecta* (or *Hyalotheca neglecta*), usually without particular comment (e.g. Grönblad, 1921; Scott *et al.*, 1965; Bourrelly, 1966) and sometimes by indicating it as a "forma" or separate variety. Bourrelly (1957, p. 1094) distinguished two different forms within Sudanese material of *G. neglecta*. Whereas his "forma 1" could be identified as "true" *G. neglecta*, his "forma 2" is in good agreement with our cylindrically-shaped cell material. That taxon also was reported by Bourrelly (1961, p. 351, t. 24: 4) from Ivory Coast, also as "forma". However, much earlier, Taylor (1935, p. 222, t. 49: 18) had already described *G. neglecta* var. *major*, the diagnosis of which perfectly fits the characteristics of the alga shown in Fig. 13. As its differs from "typical" *G. neglecta* not only in cell shape, but also in cell wall sculpture and zygospore morphology, I prefer to distinguish it at species level. By naming it after Pierre Bourrelly, both Bourrelly's contribution to the taxonomy of this species and his significance to desmidiology in general are expressed. In the Eskol sample investigated, zygospores of *G. bourrellyi* were common and measured 28-36 µm in greatest length.

In view of the above-discussed characteristics of *G. bourrellyi*, *G. neglecta* var. *elongata* Scott *et* Grönbl. is transferred to this species:
G. bourrellyi var. ***elongata*** (Scott *et* Grönbl.) comb. nov.
Basionym: *G. neglecta* var. *elongata* Scott *et* Grönbl. 1957, p. 48, t. 35: 17-24.

Penium exiguum W. West (Figs 28-30)

As far as known, this cosmopolitan species was only once recorded with zygospores, *i.e.* from The Netherlands, by Beijerinck (1926, p. 50, t. 9: 191). The (irregular) globular zygospores (Figs 29-30) commonly occurring in the Eskol sample agree with Beijerinck's report both in morphology and diameter (17-24 µm).

Staurastrum eskolense nom. nov. et stat. nov. (Figs 16-22)
Replaced synonym: *S. elegantissimum* Johnson var. *brasiliense* Förster 1969, p. 81, t. 46: 1-3.

The alga depicted in Figs 16-20 is in good agreement with *S. elegantissimum* var. *brasiliense* as described by Förster (1969) from the Amazon area and later on also recorded from French Guiana (Thérézien, 1985, p. 132, t. 39: 8). However, the taxon in question is distinctly different from *S. elegantissimum* as originally described by Johnson (1894, p. 290, t. 211: 16). Our entity is characterized by a cup-shaped semicell body, with an interrupted, annular series of spines and granules at its base. The spines alternate in position with radiating processes. A variable number of smaller, acute granules occur next to these spines (Figs 19-20). In contrast to that, *S. elegantissimum* as figured by Johnson (*loc. cit.*) and, e.g., Scott & Grönblad (1957) is marked by a cylindriform semicell body, ornamented with a regular, supraisthmial series of equally sized spinelets. In this feature, *S. elegantissimum* shows relationship with *S. johnsonii* W. *et* G.S. West, and *S. pseudosebaldi* Wille. Therefore, I prefer to classify var. *brasiliense* as a separate species. As the names

of *S. brasiliense* Nordst. and *S. foersteri* Coesel already exist, the species is named after the locality where it was found, *i.e.* Eskol.

S. eskolense was frequently met with in a sexual state. Zygospores were globular and furnished with long, apically furcated spines. Including spines, zygospores were 60 to 70 µm in diameter (Figs 21-22).

Xanthidium trilobum Nordst. (Figs 23-27)

To date, this species, characterized by a Latin American distribution (Coesel, 1996), was only known in vegetative state. Zygospores in the Eskol sample (Figs 26-27) were globular in shape and provided with rather long, apically furcated spines. Spores were 85-110 µm in diameter, including spines.

Unknown zygospore, type I (Figs 31-32)

According to the shape of both spores and adhering empty cell parts, as well as the absence of any visible somatic cell wall sculpturing, the algal form figured presumably belongs to the Fam. Mesotaeniaceae. However, since chloroplast morphology in the formaldehyde-fixed sample could not be studied it is an open question whether it concerns, e.g., a *Cylindrocystis* or a *Mesotaenium* species.

Unknown zygospore, type II (Figs 33-34)

The identity of this algal form is even more questionable than that of type I. The shape of the zygospore reminds that of *Closterium navicula* (Bréb.) Lütkem., but the apices of the adhering cell parts seem too broadly rounded to justify classification under that species (cf. Ruzicka, 1977). Also some *Actinotaenium* species should be considered, e.g., *A. minutissimum* (Nordst.) Teil., and *A. mooreanum* (Arch.) Teil. (cf. Ruzicka, 1981).

DISCUSSION

Next to the above-described desmid zygospores and that of the separately discussed *Mateola curvata* (Nordst.) Coes. (Coesel, 1997) some additional spores were found, obviously belonging to desmid taxa but poorly developed or secondarily deformed. Altogether, zygospores of fifteen desmid taxa were found. Remarkably, many other samples collected in the same period and in the same region (some of which were very close to the Eskol site) did not yield any zygospores. This phenomenon of simultaneous multi-species conjugation in desmids has been reported previously (e.g. Beijerinck, 1926; Homfeld, 1929; Coesel, 1974) but, so far, cannot be explained in a satisfying way. No doubt, conjugation is initiated by certain environmental parameters. For instance, it is well-known that mating in *Closterium* is stimulated by nitrogen depletion of the surrounding medium (e.g. Ichimura & Kasai, 1990). As zygospore formation in desmids is a way to survive dry environmental conditions for a shorter or longer periods, one might predict enhancement of conjugation activity in very shallow, drying-up water bodies. However, such conditions do not offer any guarantee of zygospore formation (e.g. Homfeld, 1929), and thus, environmental conditions alone do not trigger the induction of mating behaviour.

There is ample evidence that sexual reproduction in algae, like in other organisms, is under strict control of hormonal processes which, in turn, are regulated by genetically determined sex type and compatibility factors (Van den Ende, 1976). Often, complex hormonal interactions are needed before gametic fusion may be completed. Many pheromones appear to be highly specific, and thus only functional within lower taxonomic units. For the desmid *Closterium peracerosum*, certain pheromones were even shown to be strain-specific (Nojiri *et al.*, 1995; Sekimoto *et al.*, 1995). However, not all sex pheromones are that specific. In the fungal order Mucorales (Zygomycetes) non-species-specific trisporic acids are produced, inducing the formation of sexual structures in ■ variety of taxa. For instance, trisporic acids secreted by *Mucor mucedo* strongly stimulate zygospore production in *Mucor genevensis*, *Syzigites megalocarpus* and *Zygorhynchus moelleri* too (Van den Ende, 1978). Ectocarpene, a pheromone secreted by the brown alga *Ectocarpus siliculosus*, proved to be the progenitor of a whole series of C_{11} hydrocarbons which are involved as signals in the sexual reproduction of brown algae (Boland, 1995). Interestingly, the same compounds have also been found among the volatiles released during a phytoplankton bloom in a freshwater lake (Jüttner & Wurster, 1984).

Considering the above, it might be possible that under favourable environmental conditions certain desmid species known for their ready development of sexual stages, e.g. *Cylindrocystis brebissonii*, produce a non-species-specific pheromone that stimulates other, more or less related species to mating activity. Even ■ response by desmid species to pheromones produced by non-desmid charophycean algae is possible. In this way the phenomenon of simultaneous conjugation in multi-species desmid assemblies could be explained.

ACKNOWLEDGEMENTS — The author thanks Prof. H. van den Ende who was of help in providing with relevant literature on sex pheromones. Field trips in French Guiana were undertaken from Emerald Jungle Village (Montsinéry) the warden of which, Joep Moonen, kindly tipped us about potential sampling sites.

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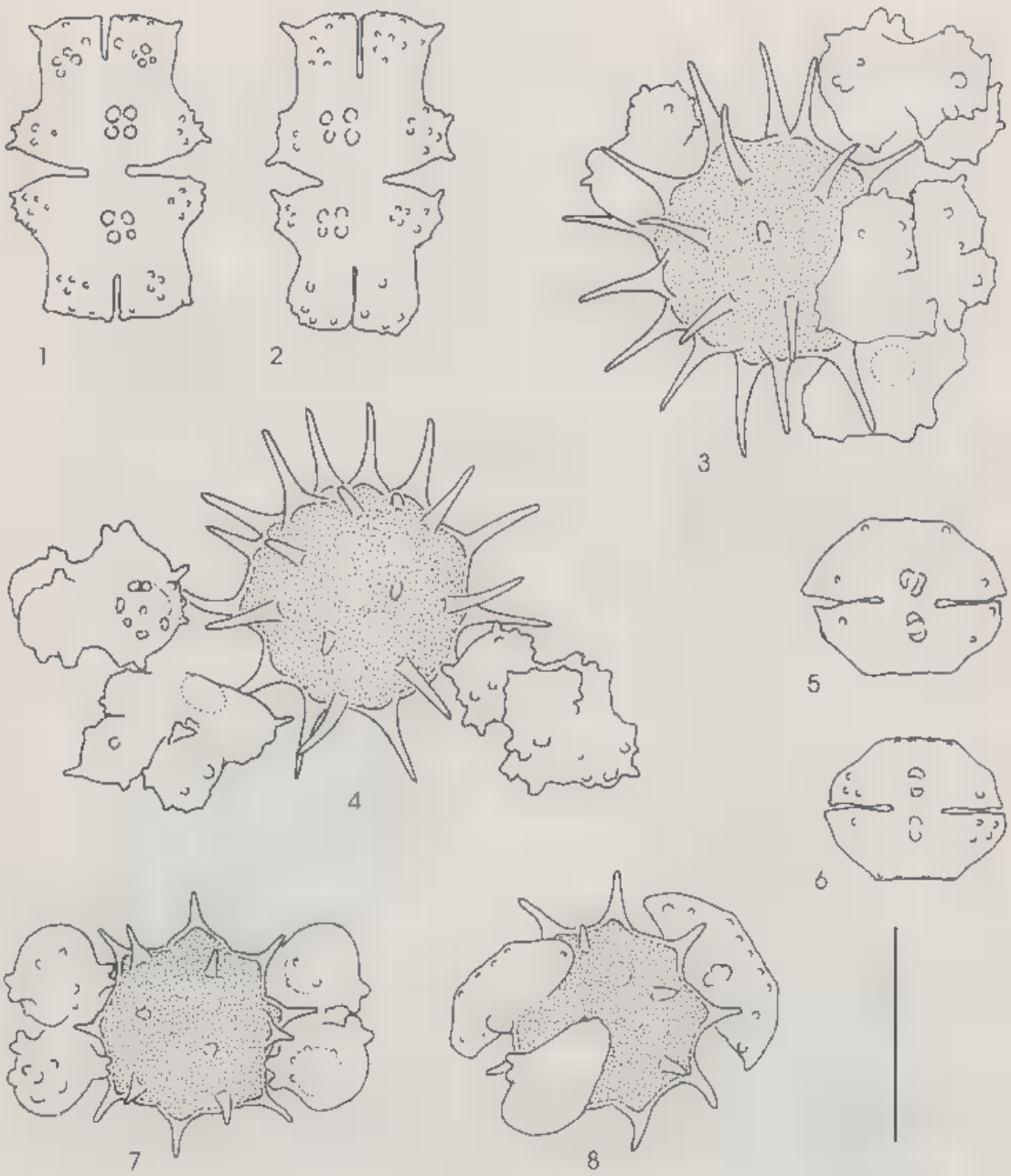
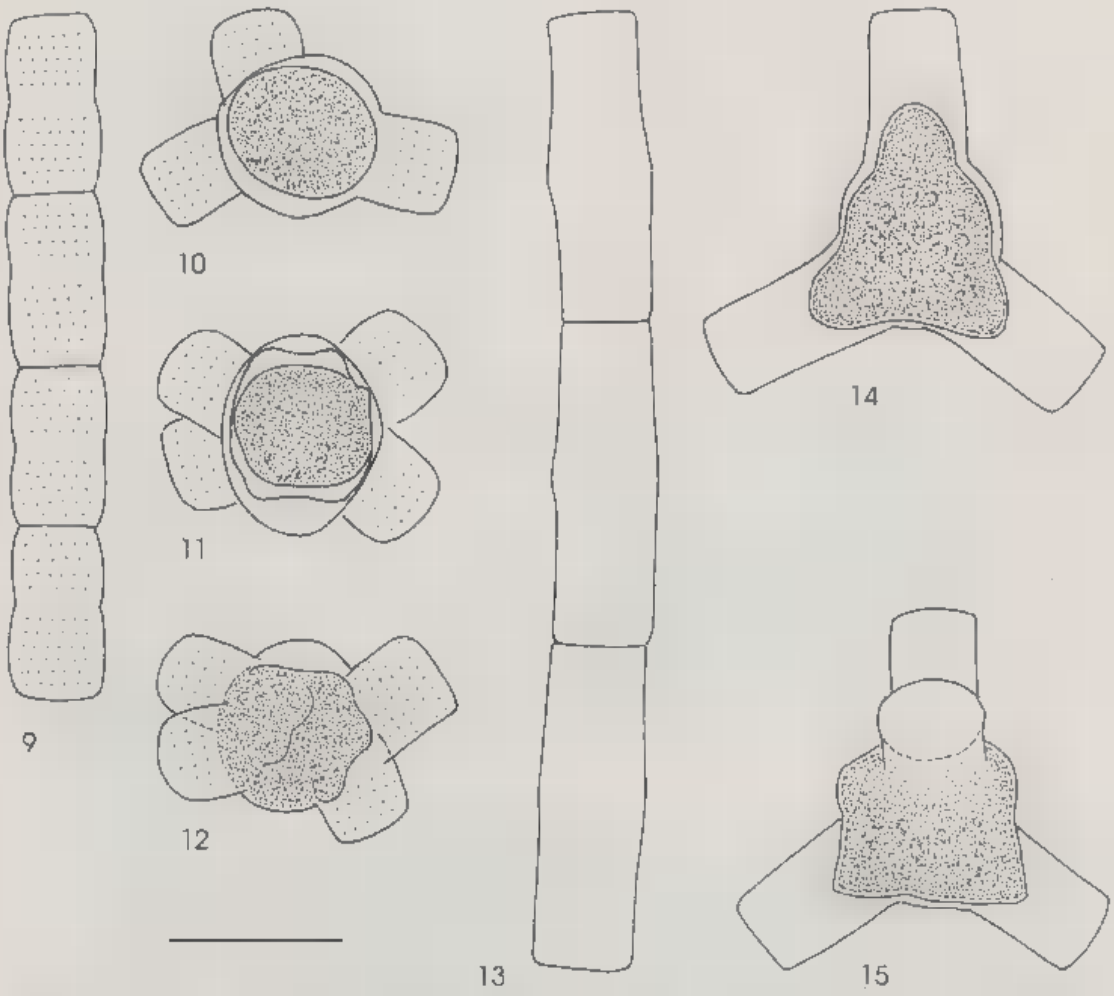
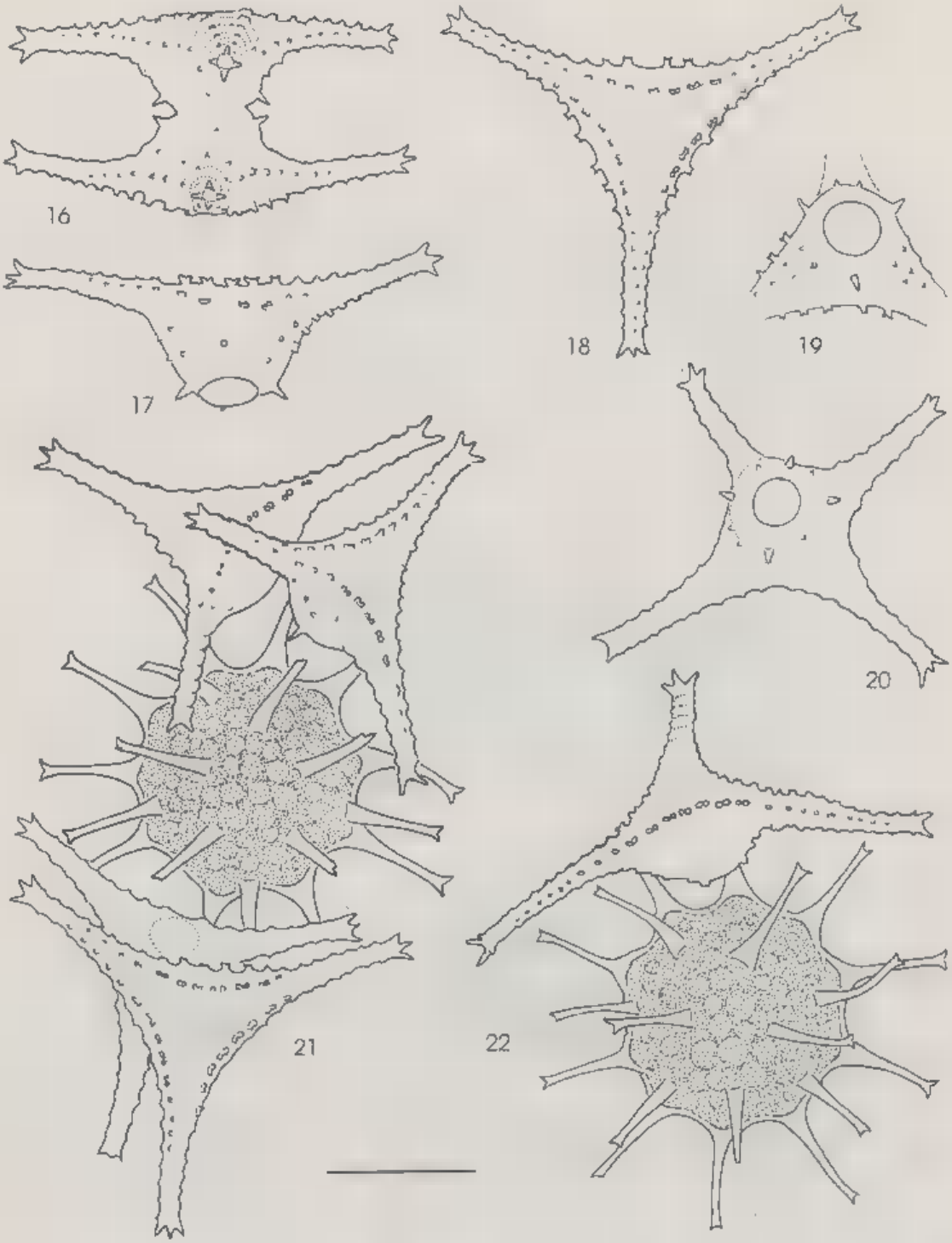


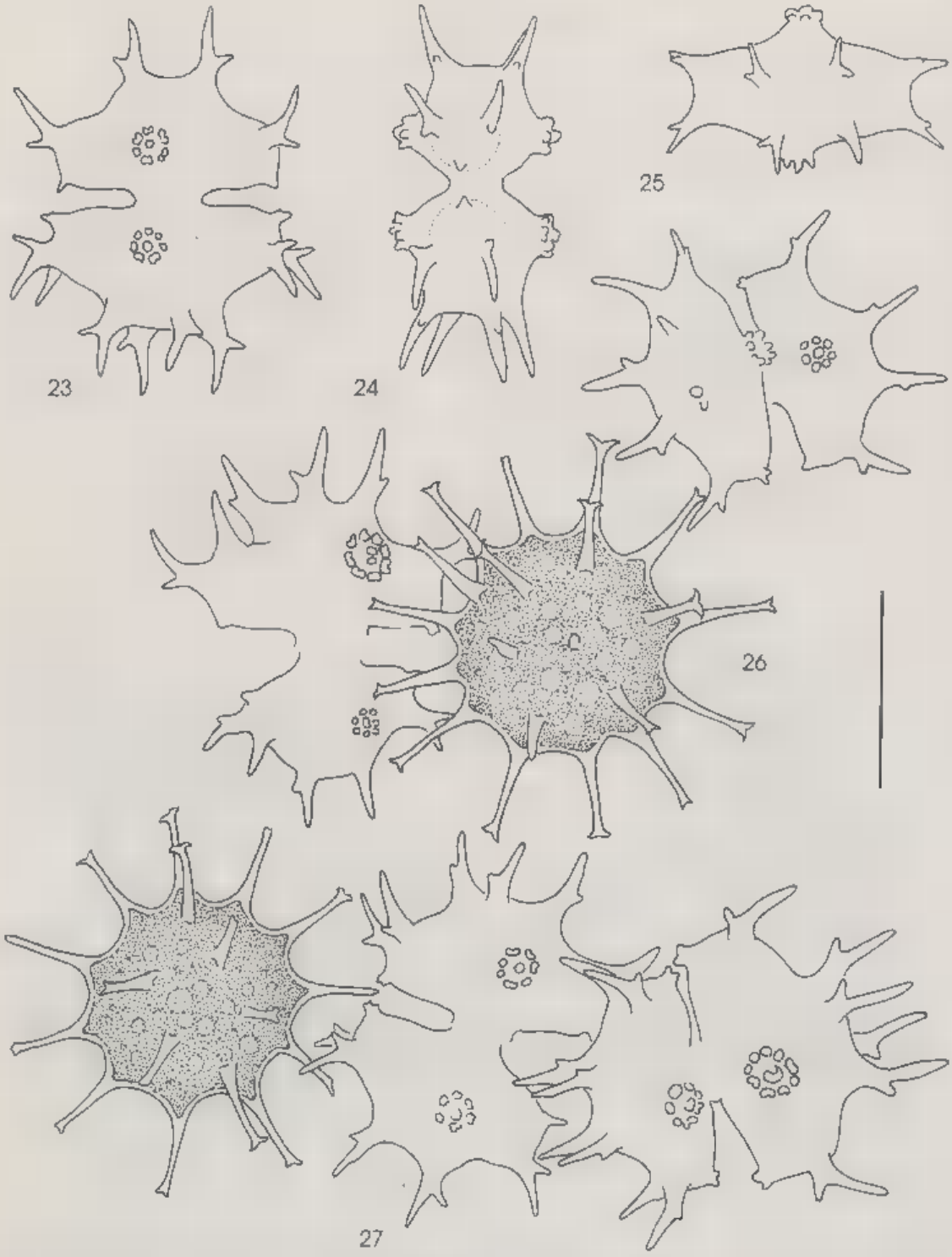
Figs 1-8. Figs 1-4. *Euastrum ciastonii* var. *apertisinuatum*. Figs 1-2. Somatic cells. Figs 3-4. Zygospores with adhering empty gametangial cells. Figs 5-8. *Cosmarium dimaziforme* var. *concavum*. Figs 5-6. Somatic cells. Figs 7-8. Zygospores with adhering empty gametangial cells. Scale bar = 25 µm.



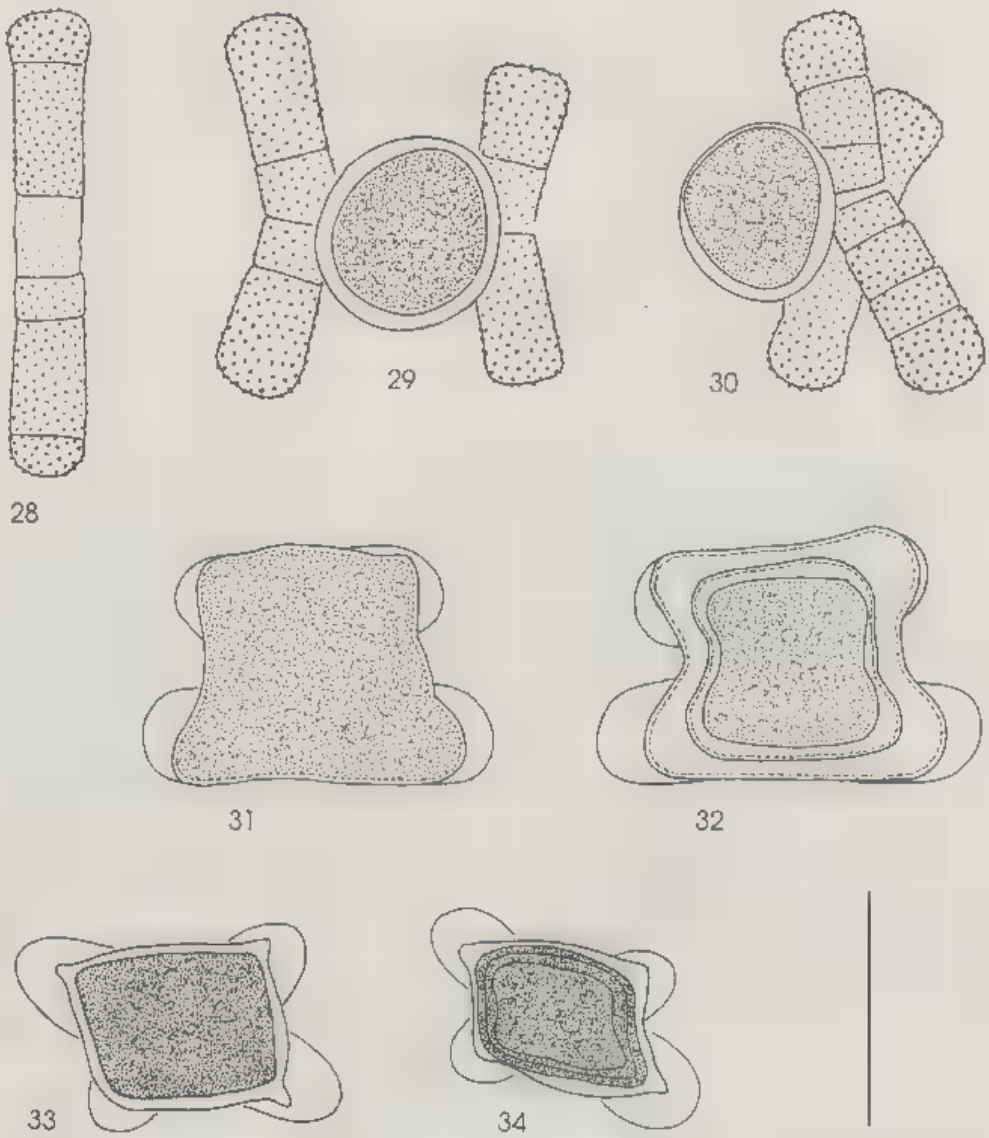
Figs 9-15. Figs 9-12. *Groenbladia neglecta*. Fig. 9. Filament of somatic cells. Figs 10-12. Zygospores with adhering empty gametangial cells. Figs 13-15. *Groenbladia bourrellyi*. Fig. 13. Filament of somatic cells. Figs 14-15. Zygospores with adhering empty gametangial cells. Scale bar = 25 µm.



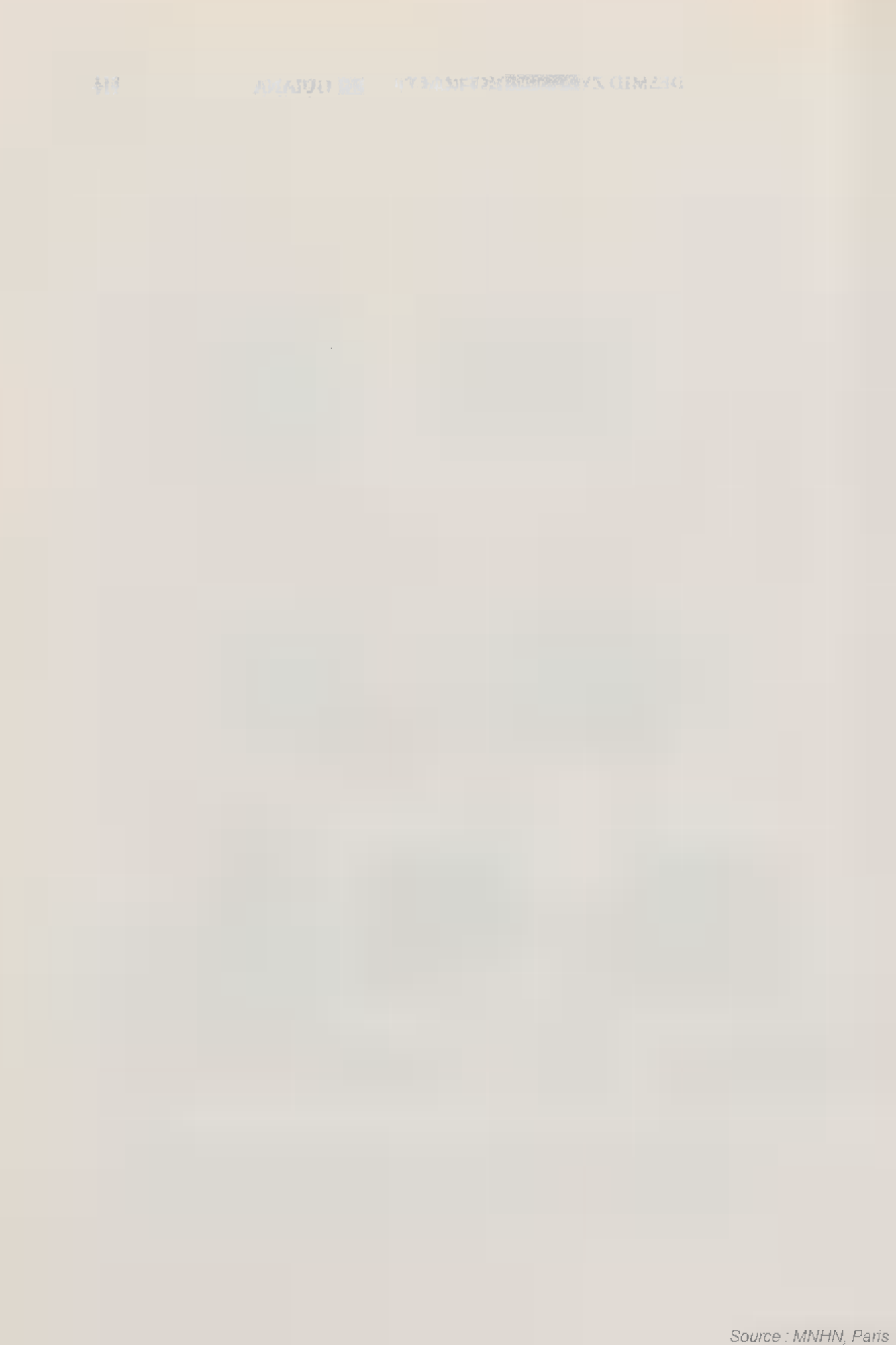
Figs 16-22. *Staurastrum eskolense*. Figs 16-20. Somatic cell/semicell in frontal view (16), back view (17), apical view (18) and isthmial view (19-20). Figs 21-22. Zygospores with adhering empty gametangial cells. (Figs 16-19, 21-22: triradiate form; Fig. 20: quadriradiate form). Scale bar = 25 µm.



Figs 23-27. *Xanthidium trilobum*. Figs 23-25. Somatic cell in frontal, lateral and apical view, respectively. Figs 26-27. Zygospores with adhering empty gametangial cells. Scale bar = 50 µm.



Figs 28-34. Figs 28-30. *Penium exiguum*. Fig. 28. Somatic cell. Figs 29-30. Zygospores with adhering empty gametangial cells. Figs 31-32. Unknown zygospore, type I. Figs 33-34. Unknown zygospore, type II. Scale bar = 25 µm.



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CONTRIBUTIONS TO THE KNOWLEDGE OF THE FRENCH DESMID FLORA 2. RARE AND REMARKABLE TAXA FROM THE REGIONS OF SOLOGNE AND BRENNE

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ABSTRACT — The examination of 32 samples collected in 1983, 1990 and 1993 in the regions of Sologne and Brenne in central France revealed a number of new, rare or otherwise noteworthy desmid taxa. In the present paper 23 taxa, belonging to the genera *Closterium* (3), *Pleurotaenium* (3), *Euastrum* (1), *Cosmarium* (13), *Staurodesmus* (1) and *Staurastrum* (2) are depicted and discussed. Three taxa are newly described: *Cosmarium boitierense* sp. nov., *C. boitierense* var. *inambitiosum* var. nov. and *C. pseudowembarense* sp. nov.; four taxa are renamed and their taxonomic status is changed: *Cosmarium berryense* nom. et stat. nov., *C. jaoi* nom. et stat. nov., *C. lutetianum* nom. et stat. nov. and *Staurodesmus reginae* nom. et stat. nov. In addition, the name of a taxon only known from North America is recombined: *Cosmarium dilatatum* var. *concavum* comb. nov.

RÉSUMÉ — L'examen de la flore desmidiale de 32 récoltes, prélevées en 1983, 1990 et 1993 dans la Sologne et la Brenne, au centre de la France, a permis de mettre en évidence un certain nombre de taxons nouveaux, rares ou autrement remarquables. Dans ce travail, 23 taxons, appartenant aux genres *Closterium* (3), *Pleurotaenium* (3), *Euastrum* (1), *Cosmarium* (13), *Staurodesmus* (1) et *Staurastrum* (2) sont identifiés et discutés. Trois taxons sont décrits comme nouveaux: *Cosmarium boitierense* sp. nov., *C. boitierense* var. *inambitiosum* var. nov. et *C. pseudowembarense* sp. nov.; quatre taxons reçoivent un nouveau nom et voient leur statut taxinomique modifié: *Cosmarium berryense* nom. et stat. nov., *C. jaoi* nom. et stat. nov., *C. lutetianum* nom. et stat. nov. et *Staurodesmus reginae* nom. et stat. nov. En outre, le nom d'un taxon connu seulement d'Amérique du Nord fait l'objet d'une nouvelle combinaison: *Cosmarium dilatatum* var. *concavum* comb. nov.

KEY WORDS: Brenne, desmids, France, freshwater green algae, Sologne, taxonomy.

INTRODUCTION

The regions of Sologne and Brenne are situated in the heart of France. Sologne is bordered on the north by the river Loire and on the south by the river Cher, covering part of the "départements" Loiret, Loir-et-Cher and Cher. Brenne lies south of Sologne, in the

"département" Indre. It forms the south-west of the old province of Berry, and is bordered on the north by the river Indre, stretching southward to the river Creuse and somewhat beyond. The major part of Brenne is designated a regional park in 1989.

Both the regions of Sologne and Brenne contain hundreds of smaller and larger ponds and lakes (French: "étangs"). Most of the lakes are artificial: they were created for fish-farming by monks in the middle ages. Situated on tertiairy sands and loam, the lakes in both regions have much in common, chemically as well as phycologically. However, few papers have been published on the algal flora, and more specifically on the desmids of these lakes (Allorge & Lefèvre, 1931; Lefèvre & Arlet, 1943; Lefèvre & Wurtz-Arlet, 1948; Wurtz, 1947, 1948).

In 1983, 1990 and 1993, as part of my studies into the desmid flora of France, I had the opportunity to collect a total of 32 samples on 29 different localities scattered over Sologne and Brenne. Although many lakes were eutrophied, desiccated or otherwise had become unfit for the development of a rich desmid flora, a total of 220 desmid taxa were recorded. In the present paper 23 remarkable taxa, occurring in one or more samples originating from 23 different localities (8 in Sologne and 15 in Brenne) are depicted and discussed taxonomically.

MATERIALS AND METHODS

The samples originate from different sites and habitats and details are given in Tables 1 and 2. The algal material was collected by squeezing out the dominant aquatics and mosses. Shortly after sampling it was fixed with formaldehyde to a final concentration of about 4%. Material was studied light microscopically, and drawings were made with the aid of a drawing tube. New reports for France are marked with an asterisk (*) in front of the species name.

OBSERVATIONS

Closterium exiguum W. & G.S. West
(Figs 3, 4)

This rare and poorly known desmid was encountered in small numbers in samples from two localities: Étang du Briou (S5) and Étang de l'Épineau (B15).

The cells are regularly and rather strongly curved, with very narrow apices without (visible) end-pore. Cell dimensions are: length 70-84 µm, breadth 4.4-4.6 µm.

Our material agrees rather well with the original description of this taxon by W. & G.S. West (1902) from paddy-fields on Ceylon. However, the taxonomy of *C. exiguum* is unclear. It resembles *C. acutum* Brébisson *in* Ralfs var. *variabile* (Lemmermann) W. Krieger, which on an average is longer and comparatively narrower. In addition, last mentioned taxon is generally irregularly curved to sigmoid. Krieger (1935) regards *C. exiguum* synonymous with *C. parvulum* Nägeli var. *angustum* W. & G.S. West which, according to Ruzicka (1977), in turn might not be related to *C. parvulum*. *C. parvulum* var.

angustum was also encountered in the study area (see below). It co-occurred with *C. exiguum* in Étang de l'Épineau, but was clearly distinguishable.

C. exiguum has previously been reported from France by Laporte (1931), from the oligo-mesotrophic Lac de Cazaux-Sanguinet-Biscarosse (Dépt Landes), unfortunately without a figure. The few remaining records of *C. exiguum* available denote it as an acidophilous taxon, which is in striking contradiction with the present finds. However, the identification of *C. exiguum* in these papers is subject to doubt (e.g., Grönblad, 1934; Tomaszewicz, 1973, 1988) and more finds are needed.

Closterium parvulum Nägeli var. ***angustum*** W. & G.S. West
(Fig. 2)

Cells attributed to this uncommon taxon were encountered in small numbers in samples from Étang de Grandelle (B6), Étang Alcoa (B7), a pool near Rosnay (B10) and Étang de l'Épineau (B15).

The cells are rather strongly curved and gradually tapering towards the narrowly rounded apices which are provided with a small end-pore. Cell dimensions are: length 106-110 µm, breadth 7.0-7.7 µm.

The present material agrees well with the description in Ruzicka (1977). In the original description of this taxon from England by W. & G.S. West (1900) no mention is made of the presence of an end-pore; however, later authors indicate a small but distinct pore in the narrowly rounded apex (compare, e.g., Förster, 1982). Ruzicka (1977) doubts whether this variety should be atributed to *C. parvulum*. In my opinion, the general cell shape suggests a relation with *C. parvulum*; var. *angustum* should otherwise be made a separate species. However, more detailed information on the shape of the apex is needed before a decision can be made. According to Krieger (1935) *C. parvulum* var. *angustum* would be synonymous with *C. exiguum* (see above).

This meso- eutrophic taxon was previously mentioned from Étang de Kergadoret (Dépt Morbihan) by Compère (1969), unfortunately without an accompanying figure, and from Étang Rablais (Dépt Sarthe) by Manguin (1936), with a figure very similar to the present material (see also below under *Euastrum germanicum* and *Cosmarium berryense*).

Closterium parvulum Nägeli var. ***cornutum*** (Playfair) W. Krieger
(Fig. 1)

A few specimens of this rare taxon were encountered in a sample from Étang de l'Hardouïne (B14).

The cells are strongly curved, rather broad but not swollen in the middle, and strongly tapering towards the narrowly rounded apices; the apex is provided with a small end-pore. Cell dimensions are: length 115-120 µm, breadth 20-22 µm.

These dimensions are somewhat below the lower limit given for this taxon by Ruzicka (1977; compare also Dürrschmidt, 1985). The shape of the apex and the absence of a central inflation point to a relation with *C. parvulum*.

This taxon was originally described from Australia (N.S. Wales; see Playfair, 1907), but apparently it has a world-wide distribution. However, most finds need confirmation and its ecological preferences are as yet unknown.

* ***Pleurotaenium excelsum*** (Turner) Gutwinski var. ***borgei*** (W. & G.S. West) Bando (Figs 9, 10)

Cells of a *Pleurotaenium* species were found in small numbers in a sample from Étang des Levrys (S8).

The cells are rod-like and comparatively narrow, faintly attenuating towards the very slightly inflated apex. The semicells have a distinct basal inflation and one or two smaller undulations above it. At lower magnifications, the apex appears smooth. However, at higher magnifications generally an apical or slightly subapical ring of small tubercles is visible, with up to six visible simultaneously. In some semicells these tubercles are only poorly developed and hardly discernible. Chloroplasts are most probably ribbon-like, containing numerous small pyrenoids. Some ten crystals are visible in the apical vacuole. Cell dimensions are: length 340-352 µm, breadth (at the level of the basal inflation of the semicells) 18-18.5 µm, length/breadth ratio *ca* 19.

This *Pleurotaenium* was first identified as *P. baculoides* (Roy & Bisset) Playfair. However, this last-mentioned taxon is characterized by longer and comparatively narrower cells, measuring up to 685 × 23 µm, without any apical ornamentation (Ruzicka, 1977). *P. baculoides* was originally described from Japan by Roy & Bisset (1886). Unfortunately they figure only one semicell at low magnification, measuring 265 × 15 µm (Roy & Bisset, *loc. cit.*, pl. 268: 18). *P. baculoides* was recently reported from South-West France, by Capdevielle (1979). His figure shows a cell that is similar to the forms given by Ruzicka (1977), but clearly different from the present material (Capdevielle, *loc. cit.*, pl. 8: 2). On the other hand, cells identical with the *Pleurotaenium* under discussion have been reported as *P. baculoides* by Kouwets (1987) from Lac de Bourdouze in the French Auvergne, ■ habitat rather similar to that of the present material.

The present material also resembles *P. ehrenbergii* (Ralfs) De Bary. However, the apex of *P. ehrenbergii* is never inflated and the crown of tubercules is different and never placed subapically (Ruzicka, 1977). In addition, the present cells are rather slender as compared with those attributed to the nominal variety of *P. ehrenbergii*, and too short as compared with its var. *elongatum* (W. West) W. West (Ruzicka, 1977; see also below).

In 1988, Bando published an extensive revision of *Docidium*, *Haplotaenium* and *Pleurotaenium* in which he presents three varieties of *Pleurotaenium excelsum* (Turner) Gutwinski, viz., the nominal variety, var. *borgei* (W. & G.S. West) Bando and var. *angustum* (W. & G.S. West) Bando. These varieties are characterized by elongate semicells with a prominent basal inflation and one or a few swellings above it. The semicells are slightly tapering towards the truncately rounded apex, on which 3-6 small conical tubercles are visible. The varieties are mainly differing in their cell diameter. The nominal variety, measuring 400-520 µm × 21-24 µm, is considered synonymous with *P. ehrenbergii* (Ralfs) De Bary var. *elongatum* (W. West) W. West by Bando (1988), but in my opinion these taxa in their original conception are morphologically different and should be kept separate. Var. *borgei* was originally described as a variety of *P. ehrenbergii* from Ceylon by W. & G.S. West (1902). The original drawing given by these authors (*loc. cit.*, pl. 18: 28) agrees rather well with the drawings given by Bando (1988, fig. 18: 4-7), and also with the present material. As dimensions W. & G.S. West (1902) give 368 × 19 µm, and Bando (1988) gives 280-455 µm × 16-21 µm, with a length/breadth ratio of 16.5-23.4. Even more interesting are micrographs of Bando (1988, fig. 42: 3, 4) which show cells with slightly inflated apices. The present material is therefore identified as *P. excelsum* var. *borgei*. Var. *angustum*, most curiously also described by W. & G.S. West in their same 1902 Ceylon paper, but as a variety of their *P. hypocymatium*, apparently is only a more slender form of var. *borgei*.

Ruzicka (1977) considered *P. excelsum* not indigenous in Europe. Disregarding the suggested synonymy with *P. ehrenbergii* var. *elongatum* (see above), its main area of distribution seems to be Asia, and the present find of var. *borgei* in France is the first European report. This taxon most probably is more common in similar mesotrophic habitats.

Pleurotaenium maximum (Reinsch) Lundell
(Fig. 8)

Pleurotaenium maximum was found in small numbers in a sample from Étang des Levrys (S8).

The cells are elongate and rod-shaped, only slightly attenuated towards the smooth, truncate apex. The semicells have a basal inflation, and one or two more or less prominent undulations above it. The chloroplasts have numerous small and scattered pyrenoids. Cell dimensions are: length 850-900 µm, breadth (at the level of the basal inflation of the semicells) 50-52 µm.

P. maximum is often classified as *P. trabecula* (Ehrenberg) *ex* Nägeli var. *maximum* (Reinsch) Roll (e.g. Krieger, 1937; see Ruzicka, 1977). However, the overall morphology of *P. maximum* is different from that of *P. trabecula*, and more specifically from its large varieties *crassum* Wittrock and *robustum* Hustedt, by its more cylindrical semicells (Ruzicka, 1977). Most interestingly, *P. trabecula* var. *robustum* was also encountered in the study area, but in a different habitat (see below). A taxon very similar to *P. maximum* is *P. archeri* Delponte: both taxa are generally considered synonymous (Ruzicka, 1977; compare W. & G.S. West, 1902; see, however, Bando, 1988).

This large *Pleurotaenium* apparently is very rare. It has previously been reported from France by Lemaire (1884, 1889, as *P. archeri*), Des Cilleuls (1929, from the river Loire!), Pourriot *et al.* (1969, from Étang du Brochet, Dépt Yvelines), Tassigny (1975, from various localities, among which Étang du Puits and Étang de Pommereau in Sologne), and Capdevielle (1979), but unfortunately none of these authors provided a figure. Cells very similar to the present material were encountered in a sample from the mesotrophic Étang de Balcère, in the eastern French Pyrénées (Kouwets, unpublished).

Pleurotaenium trabecula (Ehrenberg) *ex* Nägeli var. ***robustum*** Hustedt
(Figs 11, 12)

Very broad specimens of *P. trabecula* var. *robustum* were encontered in small numbers in a sample from Étang de l'Épineau (B15).

The cells are rod-shaped; the semicells have a prominent basal swelling, occasionally followed by a faint undulation directly above it. They are gradually tapering towards the smooth, truncate apex. In a few specimens the lower half of the semicells (apart from the basal swelling) is almost cylindrical. Cell dimensions are: length 460-600 µm, breadth (at the level of the basal swelling of the semicells) 63-73 µm, breadth of apex 25-30 µm.

The poorly known *P. trabecula* var. *robustum* was described by Hustedt (1911), from a ditch in the Austrian Alps (Tirol), but unfortunately without a figure. Cell dimensions were given as 362.5 × 62.5 µm. Dick (1926) was the first who published a figure of a *Pleurotaenium* attributed to this variety, and his material originated from a boggy habitat in Germany. The present forms, on the other hand, occurred in a sample

from a eutrophic, slightly alkaline lake. The figure given by Dick (*loc. cit.*, pl. 18: 5) is rather similar to the material figured in the present paper, but its dimensions only measure *ca* 320 × 52 µm.

This taxon has previously been mentioned from France by Deflandre (1929) and Laporte (1931), from bogs in the Haute-Savoie. Unfortunately, Deflandre (*loc. cit.*) didn't present a figure; the figure given by Laporte (*loc. cit.*) shows a slightly inflated semicell. Wurtz (1947) mentions *P. trabecula* var. *crassum* Wittrock from Étang Massé in Brenne, measuring 400 × 55 µm. However, according to Ruzicka (1977) var. *crassum* is generally smaller than 50 µm. In addition, the figure given by Wurtz (*loc. cit.*, fig. 19) is very similar to the present material.

Euastrum germanicum (Schmidle) W. Krieger
(Figs 5-7)

This *Euastrum* species was found as a rare element in samples from three lakes in Brenne, viz., Étang du Grand Mez (B5), Étang Alcoa (B7), and Étang de l'Épineau (B15).

The cells are circular to slightly oval in outline; the apical lobe of the semicells is rather narrow with parallel sides or only slightly dilated; the basal and lateral lobes are slightly conical and obtusely rounded. Incisions between basal and lateral lobes are right — to acute-angled; those between lateral lobes and apical lobe are similar but slightly deeper. The lobes are ornamented with rows of small conical warts which may be geminate or divided into two to four smaller granules. The centre of the semicells has an ornamentation of larger, obtuse and sometimes divided warts, generally roughly arranged in concentric circles (Figs 6, 7). In some specimens a tendency to a more linear arrangement of the inner warts of this central ornamentation is visible (Fig. 5). Cell dimensions are: length 54-60 µm, breadth 50-54 µm, thickness 28 µm.

The taxonomy of *E. germanicum* and the related species *E. spinulosum* Delponte and *E. gemmatum* (Brébisson) Brébisson *ex* Ralfs is very confused (Krieger, 1937; Coesel, 1978; Ruzicka, 1981). However, I disagree with the opinion advanced by last-mentioned author. I subscribe the opinion of Grönblad (1960), who pointed out that *E. gemmatum* is a clearly defined species not closely related to either *E. germanicum* or *E. spinulosum*. On the other hand, *E. germanicum* and *E. spinulosum* obviously are very closely related; *E. spinulosum* and its infraspecific taxa apparently have a more (sub)tropical distribution (Krieger, 1937). The many intermediate cell forms reported in literature suggest that *E. germanicum* may be regarded as a "temperate" variety of *E. spinulosum*. However, for mere practical reasons both taxa will be treated as separate species here, and the present material is referred to *E. germanicum*. The taxon originally described as *E. gemmatum* ssp. *mononcylum* by Nordstedt (1880) in consequence should better be classified as *E. spinulosum* var. *mononcylum* (Nordstedt) Gutwinski. Classification of the most interesting forms of var. *mononcylum* with two additional, weakly developed lateral humps (see, e.g., Förster, 1969) requires a closer study after new finds of rich material. The disposition of the central warts in the material from Étang de l'Épineau was similar to that in *E. germanicum* var. *bulnheimii* (Raciborski) W. Krieger (see Fig. 5). However, this character seems too trivial to justify classification as a separate variety (compare Ruzicka, 1981).

A *Euastrum* very similar to the present material has been reported under *E. spinulosum* from Lac de Grand-Lieu (Dépt Loire Atlantique) by Allorge (1924; see, however, Grönblad, 1931, who referred this form to *E. mononcylum* var. *germanicum*). Allorge & Lefèvre (1931) mentioned *E. spinulosum* from Étang de Fontenille in Sologne, unfortuna-

tely without a figure. Manguin (1936) reported this taxon with figures similar to the present material from Étang des Rablais (Dépt Sarthe). Wurtz (1947) described *E. spinulosum* (Schmidle) Krieger (*sic*!) var. *gallicum* from Étang Grand Riau in Brenne. In last-mentioned variety the granules on the lateral lobes occur up to the central ornamentation. The taxonomic value of this character, however, is questionable (compare Ruzicka, 1981). Messikommer (1957) reported *E. spinulosum* from a lake near St. André-de-Bouchoux ("les Dombes", Dépt Ain). His figure (Messikommer, *loc. cit.*, pl. 1: 2) shows a cell with clearly dilated apical lobes and rather narrow and acute angles between apical lobe and upper lateral lobes, characters indeed considered discriminative of *E. spinulosum* by Grönblad (1921).

Cosmarium angulosum Brébisson var. ***concinnum*** (Rabenhorst) W. & G.S. West
(Figs 29-40)

In samples from nearly all sites in Sologne (S5 and S8 excepted) and from sites B1-3 and 8-11 in Brenne, specimens of a small and morphologically variable *Cosmarium* were found in rather small numbers.

The semicells are more or less hexagonal; the apex is generally rather narrow but sometimes broader, straight or convex and merging into the lateral angles. The sides are straight or slightly retuse and sometimes diverging; the sinus is linear and closed. The semicells are oval in apical view, and broadly and truncately oval in lateral view. Cell dimensions are: length 15-18 µm, breadth 12-14 µm, thickness 7-8 µm.

The taxonomy of smooth-walled smaller *Cosmarium* taxa is very confused, and it is with some reserve that I attribute the present material to *C. angulosum* var. *concinnum*. However, general outline and morphological variability agree rather well with the figures of this taxon given by Grönblad (1924, pl. 2: 31-35). These figures are drawn after the original material collected by Rabenhorst (see also Kouwets, 1997). In addition, the dimensions of the present material are nearly the same as those mentioned by Grönblad (1924).

C. angulosum var. *concinnum* has previously been reported from France by several authors. However, only Manguin (1934, 1937) and Baïer *et al.* (1984) give figures, clearly representing very different taxa. Due to the taxonomical confusion, the ecology of *C. angulosum* var. *concinnum* is poorly known.

* ***Cosmarium asymmetricum*** Rich
(Figs 41-44)

Samples from nine localities (S2, 3, 5 and B3, 4, 9, 10, 12, 15) contained cells of a small but remarkable *Cosmarium*.

The cells are characterized by their asymmetrical front view: one side of the cells appears compressed. The shape of the semicells is very variable. The basal angles are rounded, the sides are concave and straight (one side) or diverging (other side), the apical angles are broadly rounded and sometimes merging into the lateral angles and the apex is more or less truncate or flattened with a central dent or notch. The semicells are subcircular in side view and broadly elliptic in apical view with a small central papilla. Cell dimensions are: (maximum) length 11-13 µm, breadth 9-11 µm, thickness 6.5 µm.

The present material is identical with specimens described from South Rhodesia (now Zimbabwe) as *C. asymmetricum* (Rich, 1935). Reports on similar, asymmetrical *Cosmarium*'s are very scarce, and only known from Africa. Slightly larger cells of *C. asymmetri-*

cum are reported by Rino (1972) from Mozambique. A second, larger asymmetrical species was described from West-Africa by Brandham (1967) as *C. dolabriforme*; slightly aberrant cell forms of this taxon are mentioned by Gauthier-Lièvre (1958) and Williamson (1994) under *C. asymmetricum*.

The present find in central France is the first report of a *Cosmarium* with an asymmetrical front view outside the tropics (*C. obliquum* Nordstedt, a species from upland and arctic-alpine areas, is asymmetrical in the apical view). It would be very interesting to know whether the occurrence of *C. asymmetricum* in Sologne and Brenne is just an accident (due to transport by waterfowl?), or whether the species is more common in similar eutrophic habitats in Western Europe.

Cosmarium berryense nom. et stat. nov.
Replaced synonym: *Xanthidium robinsonianum* Archer var. *divergens* Grönblad, 1938, *Bot. Not.* 1938, p. 52, fig. 8 (original description and figure)
(Figs 22, 23)

A small but characteristic *Cosmarium* was found in rather small numbers in samples from 12 different localities, including one in Sologne (Étang de la Boitière, S2) and most of the localities sampled in Brenne (B1, 3-10, 14, 15).

The cells are slightly longer than broad with a closed sinus. The semicells are hexagonal in outline, witht the lower part of the sides diverging and crenate, the lateral angles slightly protruding and bicrenate, and the upper part of the sides strongly concave. The apex is straight and 4-undulate, including the crenate apical angles. Crenations have short rows of small granules, and the apex has four intramarginal granules. The centre of the semicells is provided with two horizontally arranged small granules. The semicells are broadly oval in apical view and only faintly tumid at the level of the central papillae; they are subcircular in side view. Cell dimensions are: length 20-22 µm, breadth 18-21 µm, thickness 10-11 µm.

Searching the literature, a number of figures were found of specimens that are very similar to the present material. Most of these cell forms are attributed to *C. humile* (Gay) Nordstedt. Manguin (1936) reported a "*C. humile*, forma?" from the calcareous Étang des Rablais (Dépt Sarthe; see also above under *Euastrum germanicum*). His figures differ from the present material only in the presence of one central wart on the semicells instead of two small granules (Manguin, *loc. cit.*, pl. 4: 57-58). Huzel (1936, pl. 11: 6-8) reported a rather similar form as *C. humile* var. *subdanicum* Schmidle forma, from mesotrophic habitats in South-West Germany. Grönblad (1938) described *Xanthidium robinsonianum* var. *divergens* from South-East Finnland. His figure shows front and apical views of a semicell that are very similar to the present material, with a central ornamentation of three small granules (Grönblad, *loc. cit.*, figs. 8a-b). Finally, in 1960 Grönblad figured a *C. humile* forma from rather eutrophic habitats in Italy. The central wart in his material apparently is divided into four small granules (Grönblad, *loc. cit.*, pl. 6: 130-131; pl. 13: 6 [micrograph]). In addition, Grönblad (*loc. cit.*) figured ■ *C. garrolense* that is very similar to the material described in the present paper under *C. jaoi* (see below).

General cell shape and ornamentation suggest that the form under discussion is not closely related to *C. humile* or one of its varieties (compare Schmidle, 1896), nor to *Xanthidium robinsonianum* (compare, e.g., W. & G.S. West, 1912), and it should therefore be classified as a separate species. However, raising the variety described by Grönblad (1938) in rank and transferring it to the genus *Cosmarium* would lead to a later homonym of *C. divergens* W. Krieger. Therefore it is proposed to give the present form the new name *C. berryense*, after the old name of the region "Berry" (see the introduction of this paper).

This species obviously is widely distributed but very rare, preferring meso-eutrophic habitats.

Cosmarium boitierense sp. nov.
(Figs 53-55)
Cosmarium boitierense var. **inambitiosum** var. nov.
(Figs 56-62)

In samples from all but 7 of the localities mentioned in the present paper, cells of two very similar small *Cosmarium*'s were found in varying numbers. The two forms obviously are closely related and mainly differed by their cell dimensions. Each of the two *Cosmarium* forms was found on 9 localities: the larger cell form (see Figs 53-55) on S1-3, S5, B3, B4, and B8-10, and the smaller cell form (see Figs 56-62) on S2, S6-7, B3, B6-7, B12-13 and B15. On two localities (S2 and B3) both forms co-ocurred. The following diagnosis applies to both forms:

The cells are slightly longer than broad, moderately constricted, with a closed sinus. The semicells are basically hexagonal but with a very variable outline. The basal angles are obtuse to broadly rounded, the lower parts of the sides are straight or weakly concave and diverging, and the upper parts generally concave and converging towards a broadly truncate apex or, occasionally, merging into a convex apex. The apex has a central dent which is frequently flanked on either side by an additional undulation. In apical view the cells are elliptic with broadly rounded angles, the larger cells with a small papilla, the smaller cells with a more or less prominent central protuberance at the centre of each side of the semicells. The semicells are (sub)circular in side view. Cell dimensions of the larger specimens are: length 14-17 µm, breadth 13-15 µm, thickness *ca* 8 µm, and of the smaller specimens: length 11-14 µm, breadth 10-11.5 µm, thickness 6-8 µm.

The larger *Cosmarium* specimens agree rather well with a taxon reported under *C. subtransiens* Croasdale forma by Coesel (1991). However, Coesel (*loc. cit.*) questioned the identification of his material and suggested that it might rather be described as a new species. Similar cells are also given under *C. quadratulum* (Gay) De Toni (see, e.g., Insam & Krieger, 1936, pl. 3: 13). However, the concept of *C. quadratulum* and its infraspecific taxa is very confused (compare Coesel, 1984).

Therefore, the two *Cosmarium* forms under discussion should better be described as new taxa:

The larger specimens:
Cosmarium boitierense Kouwets var. ***boitierense***
Diagnosis: *Cellulae parvae, longitudine latitudinem paulum superante, sinu profunde et clauso. Semicellulae hexagonales, partibus inferioribus marginum lateralium divergentibus, partibus superioribus convergentibus; ambae partes rectae aut concavae. Apex leviter convexus, in media parte retusus lateribus saepe undulatis. Semicellulae a vertice visae ellipticae angulis valde rotundatis, papilla mediana parva instructae; a latere visae (sub)circulares.*
Dimensiones: longitudo 14-17 µm, latitudo 13-15 µm, crassitudo 6-8 µm.
Holotypus: figura nostra 53

The smaller specimens:
Cosmarium boitierense var. ***inambitiosum*** Kouwets
Diagnosis: *Varietas dimensionibus minoribus atque tuberculis medianis distinctis a varietate nominata differt.*

Dimensiones: longitudo 11-14 µm, latitudo 10-11.5 µm, crassitudo 6-8 µm.
Holotypus: figura nostra 56

The species is named after one of the localities where both varieties occurred together: Étang de la Boitière (S2); the varietal name *inambitiosum* means modest. Both taxa most probably have a much wider distribution in meso — eutrophic habitats.

* ***Cosmarium dilatatum*** Lütkemüller *in* Järnefeld & Grönblad
(Figs 26, 27)

Characteristic cells of *C. dilatatum* were found in rather small numbers in a sample from Étang de Paris (S1).

The morphology of the cells agrees very well with that of the material recently reported from The Netherlands by Coesel (1989), including the doubled central protrusion. The semicells are (sub)rectangular to inverted trapezoid, with extracted apical angles provided with a conical spinule. The apex is generally convex with a central excavation flanked by two intramarginal granules. A variable ornamentation of small granules is present near the basal and apical angles. Cell dimensions are: length 8.5-9 µm, breadth 8.5-11 µm, thickness 5-5.5 µm.

A *Cosmarium* obviously related to *C. dilatatum* was described by Sieminska (1965) from a pool in Montana, U.S.A., as *C. cymatonotophorum* var. *concavum*. However, cell morphology clearly suggests that this variety is not related to *C. cymatonotophorum* (compare, e.g., Kouwets, 1991), and should better be transferred to *C. dilatatum*:

C. dilatatum var. ***concavum*** (Sieminska) Kouwets comb. nov.
Basionym: *C. cymatonotophorum* W. West var. *concavum* Sieminska, 1965, *Trans. Amer. Microsc. Soc.* 84, p. 109, pl. 3: 21-25.

The nominal variety of *C. dilatatum* is not known from North America (Prescott *et al.*, 1981). As already remarked by Coesel (1989), *C. dilatatum* forms a morphological link between smaller *Cosmarium* and *Euastrum* species (compare, e.g., *E. ornans* Förster, in Förster, 1969). *C. dilatatum* apparently prefers larger, meso - eutrophic water-bodies; it most probably has a much wider distribution than presently known.

Cosmarium haynaldii Schaarschmidt
Synonym: *Cosmarium decachondrum* Roy & Bisset
(Fig. 16)

A few cells of this very rare species were found in a sample from Étang de Grandeffe (B6).

In front view the cells are more or less truncate circular with an undulating outline. Within the apical margin a row of 6 conical warts is visible; additional warts are generally present near the basal angle. I side view the semicells are circular with a flattened apex. The apical view is elliptic, showing three vertical ridges at the centre, which are not visible in front view, and two prominent conical warts at the basal angle. Dimensions of the depicted specimen are: length 30 µm, breadth 30 µm, breadth of isthmus 9 µm, thickness 17 µm.

The present material in the first instance was determined as *C. decachondrum*. This species was originally described from Japan by Roy & Bisset (1886). It has been reported from South-West France by Capdevielle (1982), and has also been found in The Netherlands (Coesel, 1991). However, Schaarschmidt (1883) had described *C. haynaldii* from Hungary, which species apparently is identical with *C. decachondrum*. Consequently the epithet *haynaldii* has priority. Raciborski (1889) classified both *C. decachondrum* and *C. haynaldii* as varieties under *C. taxichondrum* Lundell, together with two other taxa (compare also Grönblad, 1962; Grönblad & Croasdale, 1971).

In my opinion, the form under discussion shows only a superficial morphological resemblance to *C. taxichondrum* and should better be classified as a separate species: *C. haynaldii*. The many, mostly tropical varieties attributed to *C. taxichondrum* need re-evaluation. *C. haynaldii* apparently has a wide but scattered distribution in more or less mesotrophic habitats.

Cosmarium jaoi nom. et stat. nov.
Synonym: *Cosmarium garrolense* Roy & Bisset var. *crassum* Jao, 1949, *Bot. Bull. Acad. Sinica* 3, p. 51, pl. 1: 38 (original description and figure)
(Figs 13-15)

A *Cosmarium*, in the first instance identified as *C. garrolense* var. *crassum*, was found in small numbers in samples from Étang du Grand Mez (B5), Étang de la Cure (B9) and a pool near Rosnay (B10).

The cells are broadly oval in outline; the cell wall is weakly undulating with 5 undulations between basal and apical angle; the apex is truncate, the sinus is closed. Side and apical views of the semicells are broadly oval. Some cells show the presence of a row of faint intramarginal granules along the sides. Cell dimensions are: length 42-47 µm, breadth 32-36 µm, thickness *ca* 21 µm.

The present specimens agree well with the original description of *C. garrolense* var. *crassum* from China by Jao (1949). This taxon has also been reported from France by Capdevielle (1985) and from The Netherlands by Coesel (1979). From Italy, Grönblad (1960) mentioned *C. garrolense*, unfortunately without additional information. Yet, his figure (micrograph) shows a cell that is very similar to the present material (Grönblad, *loc. cit.*, pl. 13: 4). See also the remarks under *C. berryense*.

However, in my opinion general cell morphology suggests that var. *crassum* is not related to *C. garrolense*, and it should better be raised in rank to that of a separate species. To avoid any possible confusion with *Cosmarium crassum* Brébisson *in* Meneghini [publication invalid according to ICBN Art. 13.1; = *Euastrum crassum* (Brébisson) Kützing *ex* Ralfs], it is proposed to name the new species after its original author Chin-Chih Jao: *Cosmarium jaoi* Kouwets.

C. jaoi apparently is a very rare but widely distributed species from meso-eutrophic habitats.

Cosmarium limnophilum Schmidle
Synonym: *Cosmarium boeckii* Wille var. *isthmolaeve* Skuja *ex* Kouwets, 1991, p. 392, pl. 5: 1-2
(Figs 17, 18)

A few cells of a *Cosmarium* identified as *C. boeckii* var. *isthmolaeve* were found in a sample from Étang du Grand Mez (B5). The original invalid publication of this combina-

tion by Skuja (1976) was validated by Kouwets (1991). However, *C. boeckii* var. *isthmolaeve* apparently is synonymous with *C. limnophilum*, described by Schmidle (1896).

The semicells are trapeziform with convex sides and a straight apex. The sides including basal and apical angles are 6-undulate, the apex is 4-undulate. The cell wall is ornamented with one or two rows of faint intramarginal granules. The characteristic central ornamentation with three granules (one facing the apex, two facing the isthmus) generally is only very weakly developed and not visible in front view (compare Schmidle, 1896; Skuja, 1976). In apical view the semicells are broadly elliptic with a faint indication of the ornamentation; in side view they are circular. Cell dimensions are: length 32-36 µm, breadth 27.5-31.5 µm, thickness *ca* 18 µm.

The present material agrees very well with the material reported from South-West France by Kouwets (1991). Laporte (1931) mentioned it from a bog in the Haute-Savoie (together with *Pleurotaenium trabecula* var. *robustum*, see above), but his figures show specimens with a rather aberrant cell shape. *C. limnophilum* was also reported from The Netherlands by Coesel (1991). *C. gibberulum* var. *subdistichum*, described by Grönblad (1926) probably is also synonymous with *C. limnophilum* (see also Messikommer, 1929; Laporte, 1931).

C. limnophilum apparently is a rare but widely distributed species from meso- to slightly eutrophic habitats.

▪ ***Cosmarium lutetianum*** nom. et stat. nov.
Replaced synonym: *Cosmarium pygmaeum* Archer var. *apertum* Skuja, 1956, *Nova Acta Reg. Soc. Scient. Upsal.*, Ser. IV, 16 (3), p.213, pl. 36: 16 (original description and figures)
(Figs 24, 25)

Cells of a very small *Cosmarium* were found in small numbers in samples from two localities: Étang de Paris (S1) and a lake near Mézières-en-Brenne (B4).

The semicells are trapeziform with irregularly rounded angles which are provided with a granule. The sinus is widely dilated. In apical view the semicells are elliptic, showing two granules near each angle; in side view they are trapeziform with a truncate apex flanked by two granules. Cell dimensions are: length *ca* 7 µm, breadth 6-7 µm, thickness *ca* 3.5 µm.

This taxon was originally described from Sweden by Skuja (1956) as *C. pygmaeum* var. *apertum*. The apical view induced Förster (1981) to transfer this variety to *C. sphagnicolum* W. & G.S. West. However, in my opinion the general cell morphology suggests that the taxon under discussion should better be raised in rank to that of a separate species. To avoid creation of a later homonym of *C. apertum* Turner, a new name must be chosen, and it is proposed to name it after the first locality mentioned above (Lutetia is the old latin name of Paris).

C. lutetianum apparently is a very rare (or easily overlooked?) species from meso - eutrophic habitats: after the publication of Skuja (1956) it had never been reported again.

Cosmarium pseudowembarense sp. nov.
(Figs 45-52)

Cells of a characteristic but unknown *Cosmarium* species were found more or less abundant in samples from four localities in Brenne: a lake near Mézières-en-Brenne (B4), Étang Montiacre (B13), Étang de l'Hardouïne (B14) and Étang de l'Épineau (B15).

Cell morphology is very variable. The cells are about as long as broad or a little longer, deeply constricted, with a closed sinus. The semicells are hexagonal in outline, with the basal angles obtuse to broadly rounded. The lower part of the sides is parallel or divergent and slightly concave, straight or broadly convex. The lateral angles are truncate to broadly rounded, the upper part of the sides is strongly convergent and straight to strongly concave. The apical angles are obliquely rounded-truncate merging into the notched apex. In apical view the semicells are oval with broadly rounded angles and a more or less prominent central swelling; in side view they are subcircular. Cell dimensions are: length 12-15 µm, breadth 10-15 µm, thickness 6-8 µm, length/breadth ratio 1.04-1.17.

The prominence of the central swelling obviously depends on the shape of the semicell: characteristic semicells with the upper part of the sides concave have a conspicuous bulge on either side whereas semicells with the upper part of the sides straight to convex are more or less oval in apical view (compare Fig. 49).

In view of the very similar cell morphology the present form is considered identical with the specimens given under *C. laeve* var. *pseudooctangulare* Fritch & Rich by Coesel (1979), collected in rather similar eutrophic habitats. Cells attributed to last-mentioned taxon were recently also reported from South-West France by Kouwets (1991). They differ from the material under discussion and that in Coesel (1979) in the upper part of the sides being straight to convex instead of concave. However, classification as a variety of *C. laeve* Rabenhorst is questionable. *C. laeve* is characterized by an elliptic apical view whereas similar cell forms with a central swelling or protuberance are generally attributed to *C. wembarense* Schmidle (compare Förster, 1982). The present material differs from *C. wembarense* mainly by its lower length/breadth ratio (according to Förster, *loc. cit.*, *ca* 1.33 in *C. wembarense*), and it is proposed to describe it as a new species:

Cosmarium pseudowembarense Kouwets

Diagnosis: *Cellulae parvae, longitudine latitudinem fere aequante aut paulum superante, sinu lineari profunde constrictae. Semicellulae hexagonales angulis basalibus obtusis aut valde rotundatis, partibus inferioribus marginum lateralium paralellis aut divergentibus, obtusis aut levissime convexis, partibus superioribus valde convergentibus et concavis; angulis superioribus rotundatis, apice excavato. Semicellulae a vertice visae ovales medio utrimque tumides, a latere visae subcirculares.*
Dimensiones: longitudo 12-15 µm, latitudo 10-15 µm, crassitudo 6-8 µm, long./lat. ratio 1.04-1.17
Holotypus: figura nostra 50

C. pseudowembarense seems to prefer larger, rather eutrophic water-bodies. The cell forms described under *C. laeve* var. *pseudooctangulare* by Kouwets (1991) may be synonymous but more information on the morphological variability within large populations is needed before a conclusion can be drawn.

* ***Cosmarium sexnotatum*** Gutwinski var. ***bipunctatum*** (Woloszynska) Coesel
(Figs 19-21)

Cells attributed to this taxon were found in small numbers in samples from four localities in Brenne: Étang du Grand Mez (B5), Étang de la Cure (B9), and two sites near Rosnay (B10, 11).

The semicells are more or less reniform in outline. The sides, including basal and apical angles, are manifestly 4-undulate; the apex is straight and weakly 4-undulate. The ornamentation of the semicells is generally very weakly developed. Along the sides sometimes one or two rows of intramarginal granules are visible; just below the central undulations of the apex two slightly more prominent granules are present. The characteristic central ornamentation of three vertical ridges is not visible in front view. In apical view the semicells are broadly elliptic with a faint indication of the three ridges; in side view they are circular. Cell dimensions are: length 32-34 µm, breadth 28-29 µm, thickness *ca* 18 µm.

C. sexnotatum var. *bipunctatum* apparently is a widely distributed but rare taxon, and its ecological amplitude is poorly known. In The Netherlands it occurs in mesotrophic, slightly acid fen hollows (Coesel, 1989).

Cosmarium sp.
(Fig. 28)

This *Cosmarium* was encountered in very small numbers in a sample from a pool near Rosnay (B10).

The cells are about as long as broad, moderately constricted, with a closed sinus. The semicells are broadly pyramidate-trapeziform with rounded basal angles. The upper part of the sides is slightly retuse just under the apex; the apical angles are truncate and the apex is straight. At the basal angles and at the apical region the cell wall is ornamented with series of small granules; a row of four granules is present on either side of the isthmus. The centre of the semicells is smooth and finely punctate. The apical view is oval with a faint undulation at the centre of each side; the side view of the semicells is subcircular. The dimensions of the depicted specimen are: length 22 µm, breadth 20 µm, thickness 12 µm.

Despite extensive searching, no figures were found in the literature matching the present material. However, it does not seem appropriate to describe this *Cosmarium* as a new species here, since only very few specimes could be studied, especially as concerns the ornamentation of the cell wall. New reports of richer material are urgently needed.

Staurodesmus reginae nom. et stat. nov.
Replaced synonym: *Staurastrum dickiei* Ralfs var. *rhomboideum* W. & G.S. West fo. *minor* De Pouques, 1952, *Rev. Gen. Bot.* 59, p. 310, pl. 2: 24 (original description and figure; illegitimate name acc. to ICBN Art. 53.5: later homonym)
(Figs 63, 64)

Specimens of a *Staurodesmus* species were found in abundance in a pool northeast of Bélâbre (B8), and in fair numbers in a pool near Rosnay (B10).

The semicells are more or less rhomboid in outline with a broadly convex apex; the angles are broadly rounded and provided with a short spine; the spines are sometimes curved and strongly convergent. In apical view the semicells are triangular with slightly concave sides and tumid angles. The cell wall is furnished with a marked pore-pattern, consisting of pore-rings encircling the angles, an apical pore-ring with a central pore, three rows of pores running form the apical ring down the cell wall in between the angles, and groups of 3-4 pores at the base of the angles. Cell dimensions are: length 22-23 µm, breadth without spines 20-21 µm, length of the spines 1-3 µm.

In view of the very similar cell morphology, the present form is considered identical with the material from a pool in the "Forêt de Rambouillet" (Dépt Yvelines), attributed to *Staurastrum dickiei* var. *rhomboideum* fo. *minor* by Bourrelly (1953). This taxon was described shortly before by De Pouques (1952) from l'Étang de la Grange en Woëwre, in the "Forêt de la Reine" (Dépt Meurthe-et-Moselle and Dépt Meuse). However, several authors had previously described a "forma *minor*" of *Staurastrum (Staurodesmus) dickiei*. Huber-Pestalozzi (1928) described *Staurastrum dickiei* fo. *minor* which is not mentioned by Teiling (1967) since no figure was given. Manguin (1936) reported *Staurastrum dickiei* forma minor which is mentioned by Teiling (1967), but most probably "forma minor" is only intended as part of the description. Grönblad (1948) reported *Staurastrum dickiei* var. *rhomboideum* fo. *minor* as a figure without any diagnosis or other information; nevertheless it is mentioned by Teiling (1967). Teiling (*loc. cit.*) did not recombine the taxa mentioned above under *Staurodesmus* but included them, partly as "Formae minores" in the respective varieties of *Staurodesmus dickiei*. However, none of these taxa are identical with the present form.

Moreover, as already discussed by Bourrelly (1953), the characteristic pore-pattern renders a relation of the present material with *S. dickiei* questionable. However, as suggested by their different cell morphology, in my opinion it is also not related with other *Staurodesmus* taxa with similar pore-patterns (compare Bourrelly, 1953). Therefore, I propose to classify the form under discussion as a separate species. Since the original name was a later homonym, a new name must be chosen:*Staurodesmus reginae* Kouwets, after the name of the original sampling place (regina = queen). No further information on the ecology of this apparently rare taxon is available.

Staurastrum bloklandiae Coesel & Joosten
(Figs 66, 67)

Characteristic cells of *S. bloklandiae* were encountered in small numbers in samples from four localities: Étang de la Boitière (S2), Étang de Bièvre (S6), a pool near Rosnay (B12) and Étang de l'Hardouïne (B14)

This taxon was recently described by Coesel & Joosten (1996) after Dutch material. They also included data on the present material from the two localities in Sologne mentioned above. However, later on the taxon was also found on two localities in Brenne. This material agreed very well with the original description although some specimens were slightly smaller. Cell dimensions of the French material are: length including processes 23-33 µm, breadth including processes 25-37 µm, thickness *ca* 7 µm.

The additional finds in Brenne confirm the supposed preference of this taxon for more eutrophic water bodies (Coesel & Joosten, 1996). It undoubtedly has a far more wider distribution in France than the four localities mentioned above. As in the Netherlands, eutrophication of many water bodies in France most probably has promoted its distribution and desmid research in such habitats is urgently needed.

▪ ***Staurastrum gladiosum*** Turner var. ***delicatulum*** W. & G.S. West
(Fig. 65)

Specimens belonging to this taxon were scarcely found in samples from Étang Alcoa (B7), a pool near Rosnay (B10) and Étang de l'Épineau (B15).

In front view the semicells are depressed reniform and furnished with spines, those at the angles being rather long, slender and sometimes curved. The sinus is acute and open. In apical view the semicells are triangular with concave sides and the angles somewhat tapering. Cell dimensions of the depicted specimen are (without spines): length 35 µm, breadth 35 µm; length of the spines is up to 8 µm.

S. gladiosum was described from New Jersey, U.S.A. (Turner, 1885). Var. *delicatulum* differs from the nominal variety by its more slender and slightly curved spines, which also have a different disposition on the cell wall (W. & G.S. West, 1900). The present material agrees very well with the description and figure of *S. gladiosum* var. *delicatulum* given by Coesel (1975). As already pointed out by this author, the taxon under discussion apparently prefers a more eutrophic environment than the related and very similar *S. teliferum* Ralfs (see, e.g., Kouwets, 1987). *S. gladiosum* var. *delicatulum* had not previously been mentioned from France; reports on the nominal variety are very scarse and somewhat doubtful since none of them is accompanied by a figure (Frémy, 1930; Pourriot *et al.*, 1969; Verger-Lagadec, 1963; Verger-Lagadec & Villeret 1963; Villeret *et al.* , 1972; Compère, 1980).

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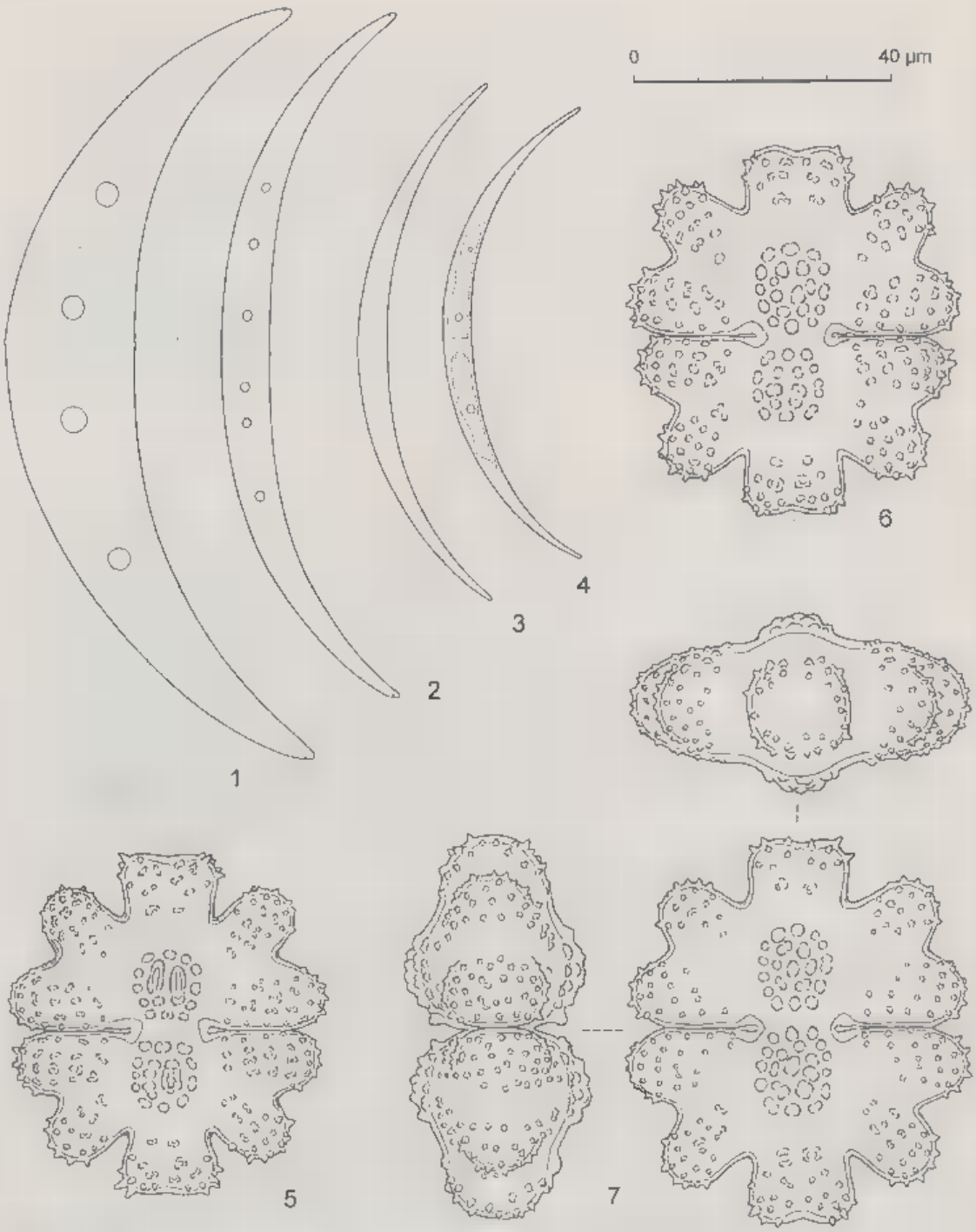
Table 1. Sampling localities situated in Sologne.

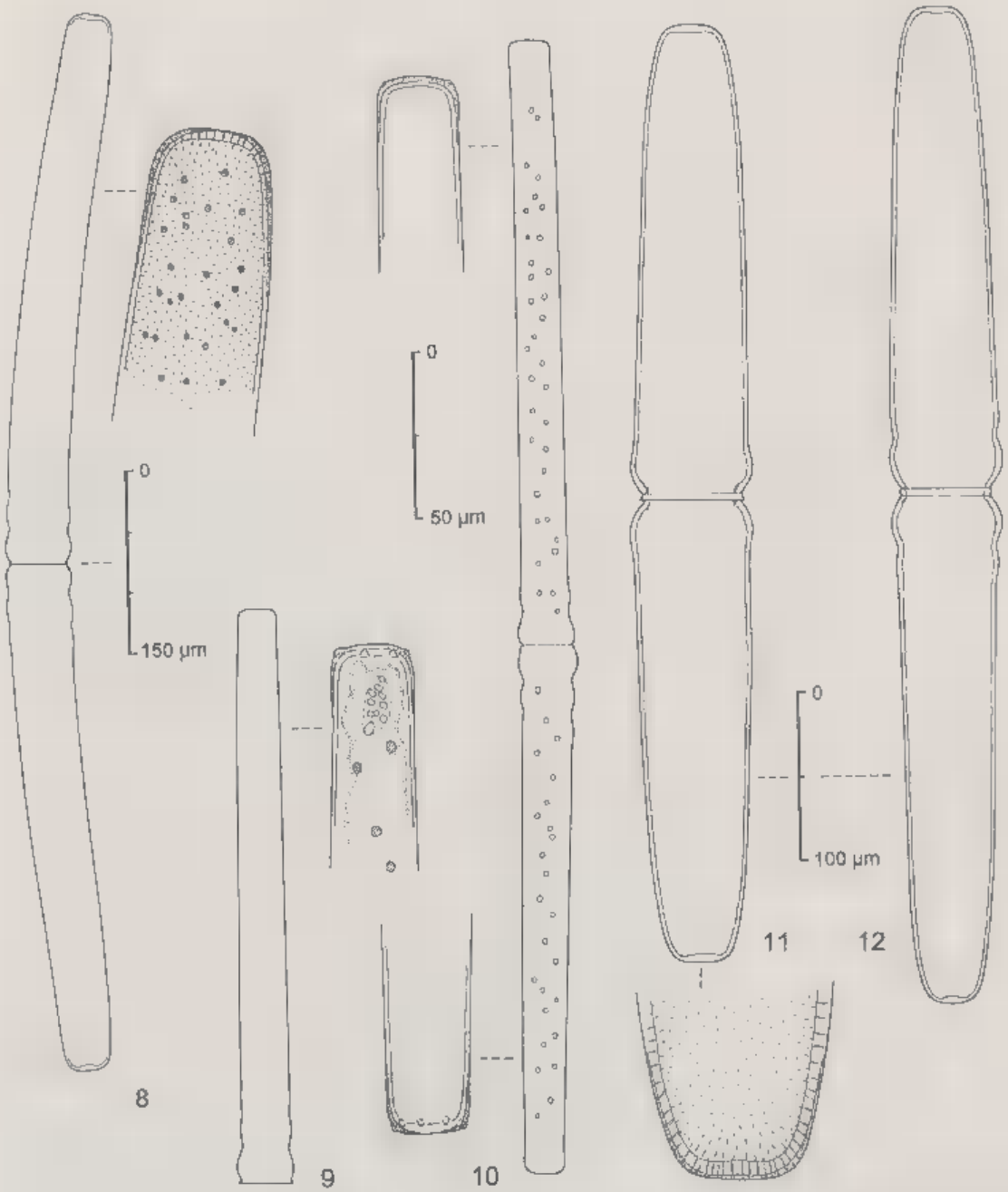
No.	Name of locality/situation	Date of sampling	Details concerning habitat and substrate
S1	Étang de Paris	22.VII.1983	Eutrophic; *Phragmites australis, Myriophyllum spicatum, Nymphaea alba*
S2	Étang de la Boitière	23.VII.1983	Eutrophic, loamy soil; *Nymphaea alba, Myriophyllum spicatum*
S3	Pool, 1.5 km east of Courmemin	23.VII.1983	Eutrophic; *Scirpus lacustris, Utricularia vulgaris*
S4	Étang de Pontbertas	23.VII.1983	Eutrophic; *Ceratophyllum demersum, Hydrocharus morsus-ranae*
S5	Étang du Briou	25.VII.1983	Eutrophic; *Phragmites australis, Iris pseudacorus, Nymphaea alba, Nuphar lutea, Trapa natans, Utricularia vulgaris*
S6	Étang de Bièvre	25.VII.1983	Eutrophic; *Najas marina*
S7	Lake, opposite Étang de Theillay	25.VII.1983	Eutrophic; *Riccia* sp.
S8	Étang des Levrys	19.VII.1990	Mesotrophic; *Hypericum elodes, Utricularia vulgaris, Nymphaea alba, Juncus bulbosus*

Table 2. Sampling localities situated in Brenne

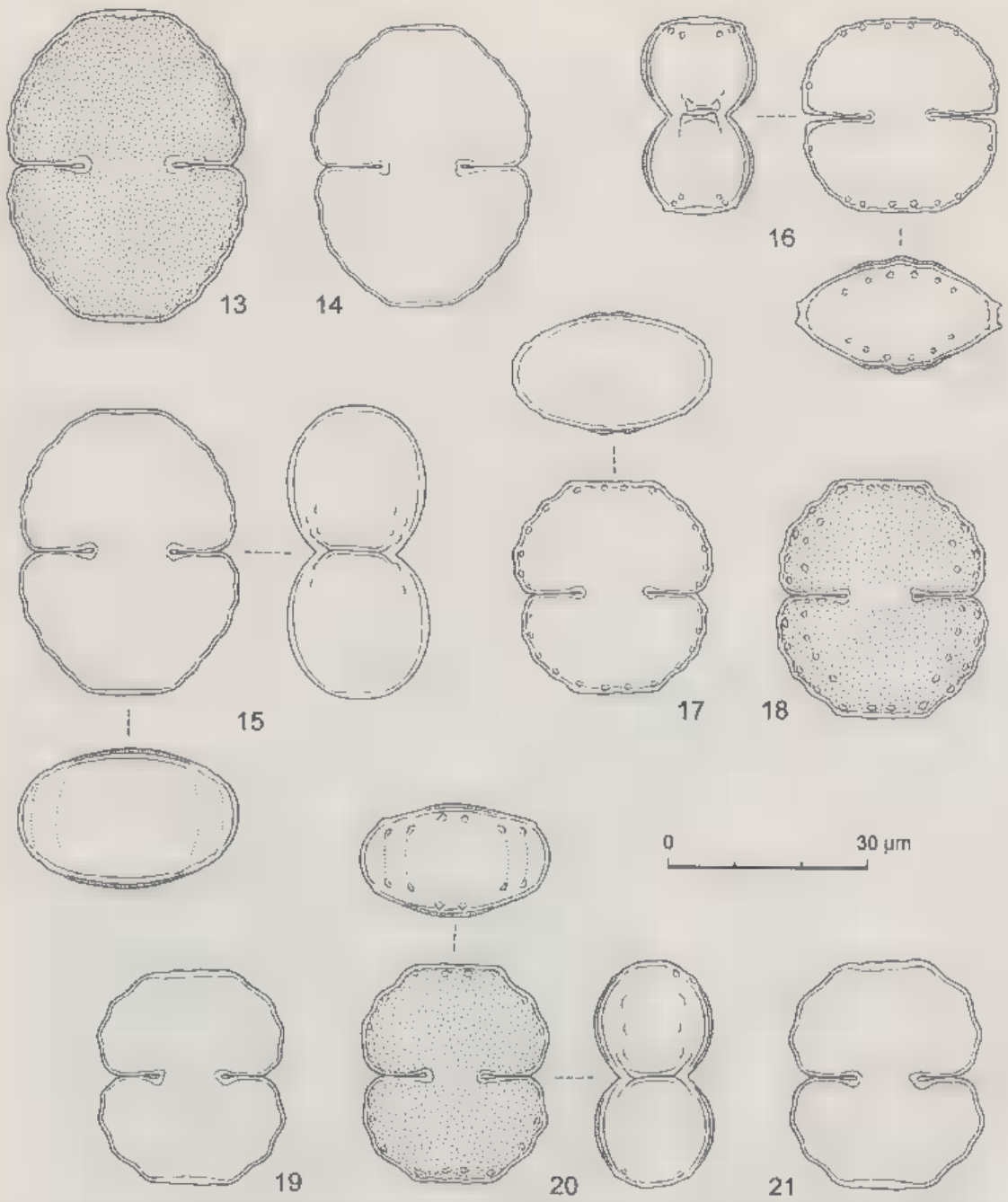
No.	Name of locality/situation	Date of sampling	Details concerning habitat and substrate
B1	Lake, 3 km west of Mézières-en-Brenne	28.VII.1983	Eutrophic; *Phragmites australis, Alisma plantago-aquatica, Typha latifolia, Myriophyllum spicatum, Potamogeton* spp.
B2	Étang des Vigneaux	29.VII.1983	Eutrophic; *Phragmites australis, Typha latifolia, Sparganium erectum, Utricularia vulgaris*
B3	Lake, 3.5 km south-east of Mézières-en-Brenne	29.VII.1983	Meso-eutrophic; *Phragmites australis, Carex* spp., *Hydrocotyle vulgaris, Scutellaria galericulata, Ceratophyllum demersum, Utricularia vulgaris*
B4	Lake, 2.5 km south-east of Mézières-en-Brenne	29.VII.1983	Eutrophic; *Phragmites australis, Utricularia vulgaris*
B5	Étang du Grand Mez	29.VII.1983	Eutrophic; *Phragmites australis, Nymphaea alba, Lythrum salicaria, Lysimachia vulgaris, Mentha aquatica, Hydrocotyle vulgaris, Carex* spp.
B6	Étang de Grandeffe	31.VII.1983	Eutrophic, loamy soil; *Phragmites australis, Typha latifolia, Nymphaea alba, Trapa natans, Myriophyllum spicatum, Utricularia vulgaris*
B7	Étang Alcoa	31.VII.1983	Eutrophic, loamy soil; *Nymphaea alba, Nuphar lutea, Potamogeton* spp., *Iris pseudacorus, Sparganium erectum, Carex* spp., *Myriophyllum spicatum, Utricularia vulgaris*
B8	Pool, 8.5 km north-east of Bélâbre	7.VIII.1993	Eutrophic, loamy soil; *Typha latifolia, Sparganium erectum, Iris pseudacorus, Potamogeton natans, Myriophyllum spicatum*
B9	Étang de la Cure	9.VIII.1993	Eutrophic, loamy soil; *Phragmites australis, Iris pseudacorus, Solanum dulcamara, Nymphaea alba, Nuphar lutea, Myriophyllum spicatum*
B10	Pool, 1.5 km north-west of Rosnay	9.VIII.1993	Eutrophic; *Phragmites australis, Nuphar lutea, Myriophyllum spicatum, Utricularia vulgaris, Najas marina*
B11	Small lake, 4.5 km north-west of Rosnay	9.VIII.1993	Eutrophic; *Phragmites australis, Lysimachia vulgaris, Lythrum salicaria, Ranunculus flammula*
B12	Pool, 4.5 km north-west of Rosnay	9.VIII.1993	Eutrophic; *Utricularia vulgaris*
B13	Étang Montiacre	9.VIII.1993	Eutrophic; *Myriophyllum spicatum, Najas marina, Potamogeton crispus*
B14	Étang de l'Hardouïne	11.VIII.1993	Eutrophic; *Phragmites australis, Myriophyllum spicatum, Utricularia vulgaris, Potamogeton* spp.
B15	Étang de l'Épineau	11.VIII.1993	Eutrophic; *Phragmites australis, Nymphaea alba, Potamogeton perfoliatus, Ceratophyllum demersum, Najas major, Myriophyllum spicatum, Utricularia vulgaris*



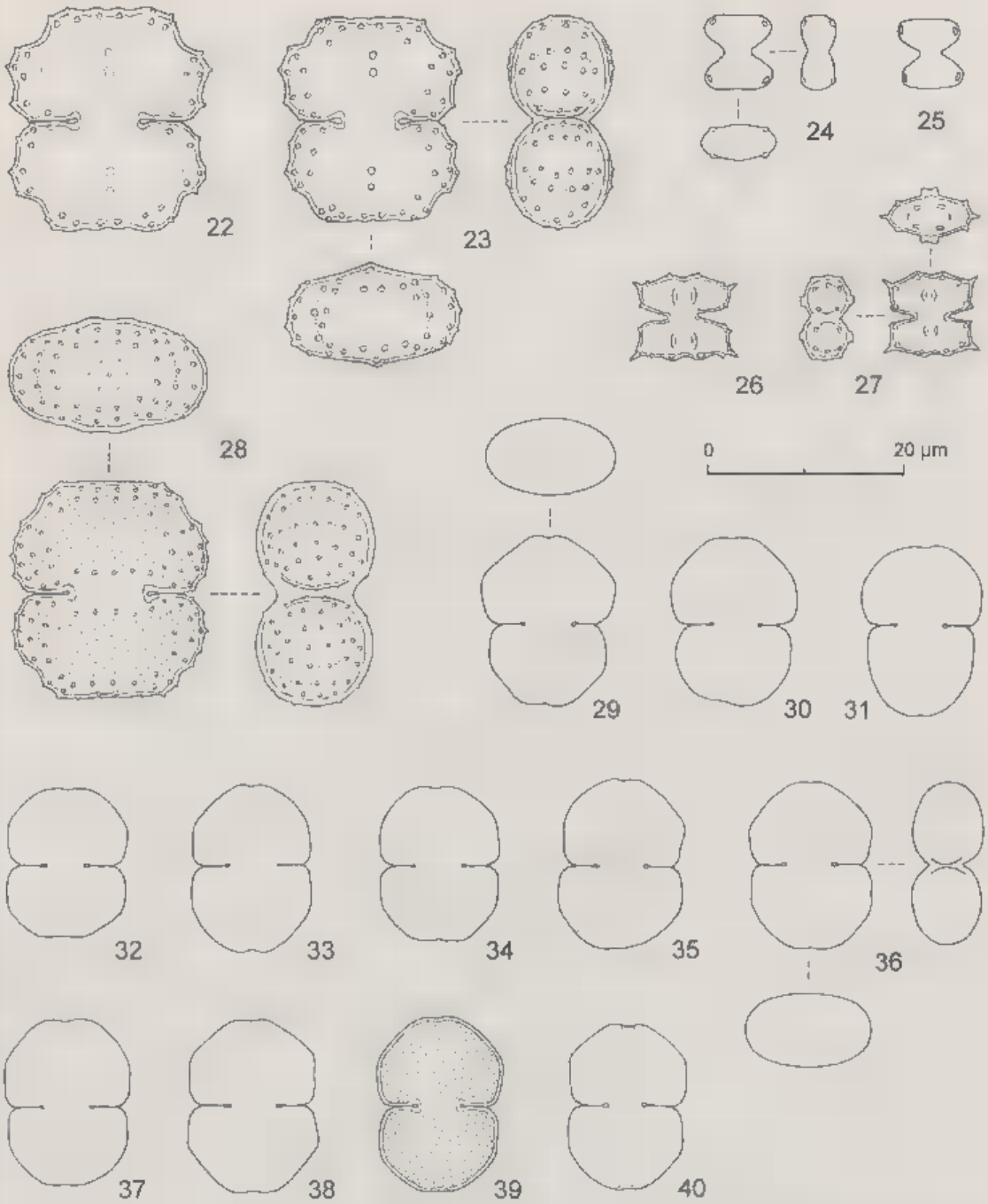
Figs 1-7. Fig. 1. *Closterium parvulum* var. *cornutum*. Fig. 2. *C. parvulum* var. *angustum*. Figs 3-4. *C. exiguum*. Figs 5-7. *Euastrum germanicum*.



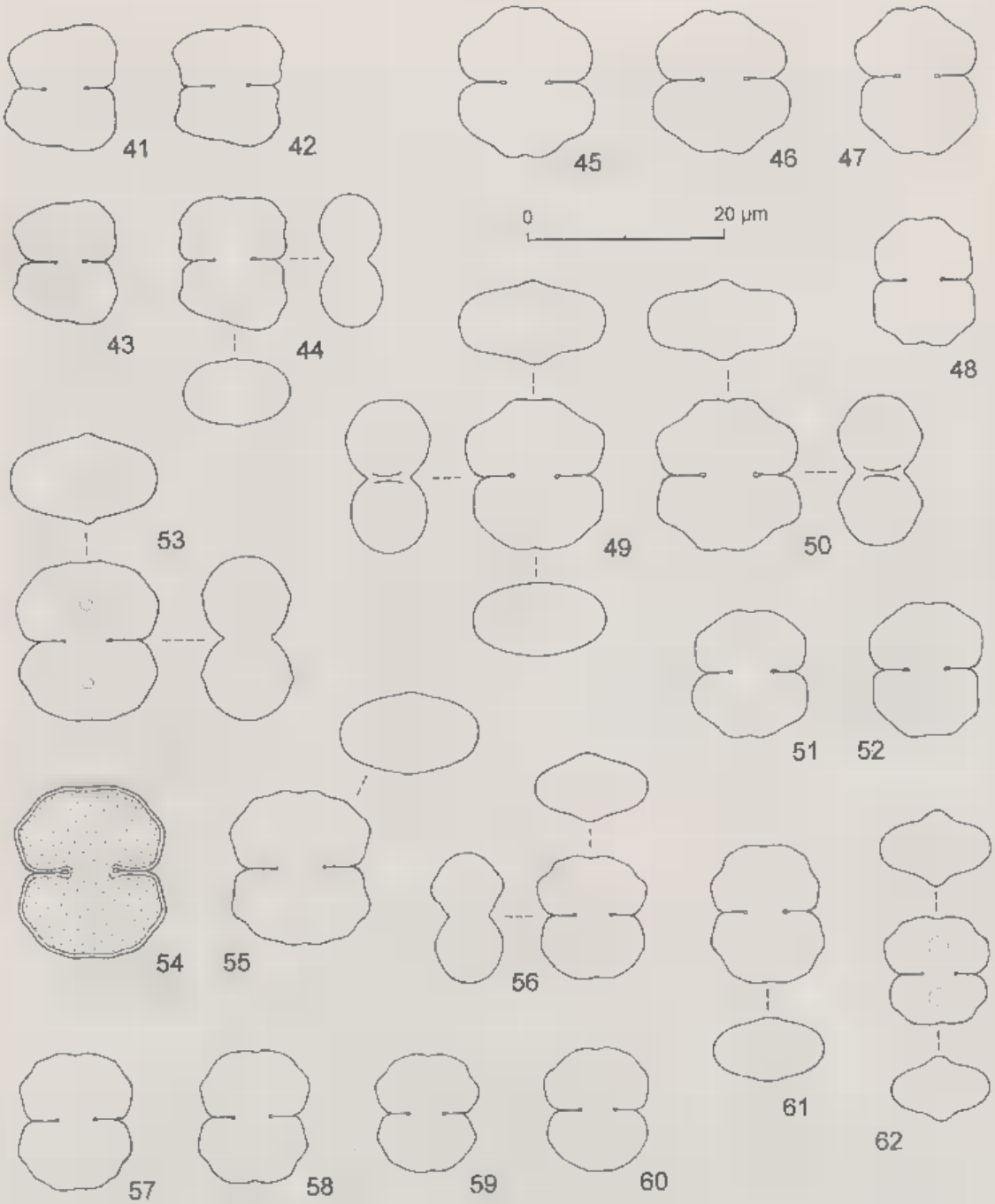
Figs 8-12. Fig. 8. *Pleurotaenium maximum*. Figs 9-10. *P. excelsum* var. *borgei*. Figs 11-12. *P. trabecula* var. *robustum*.



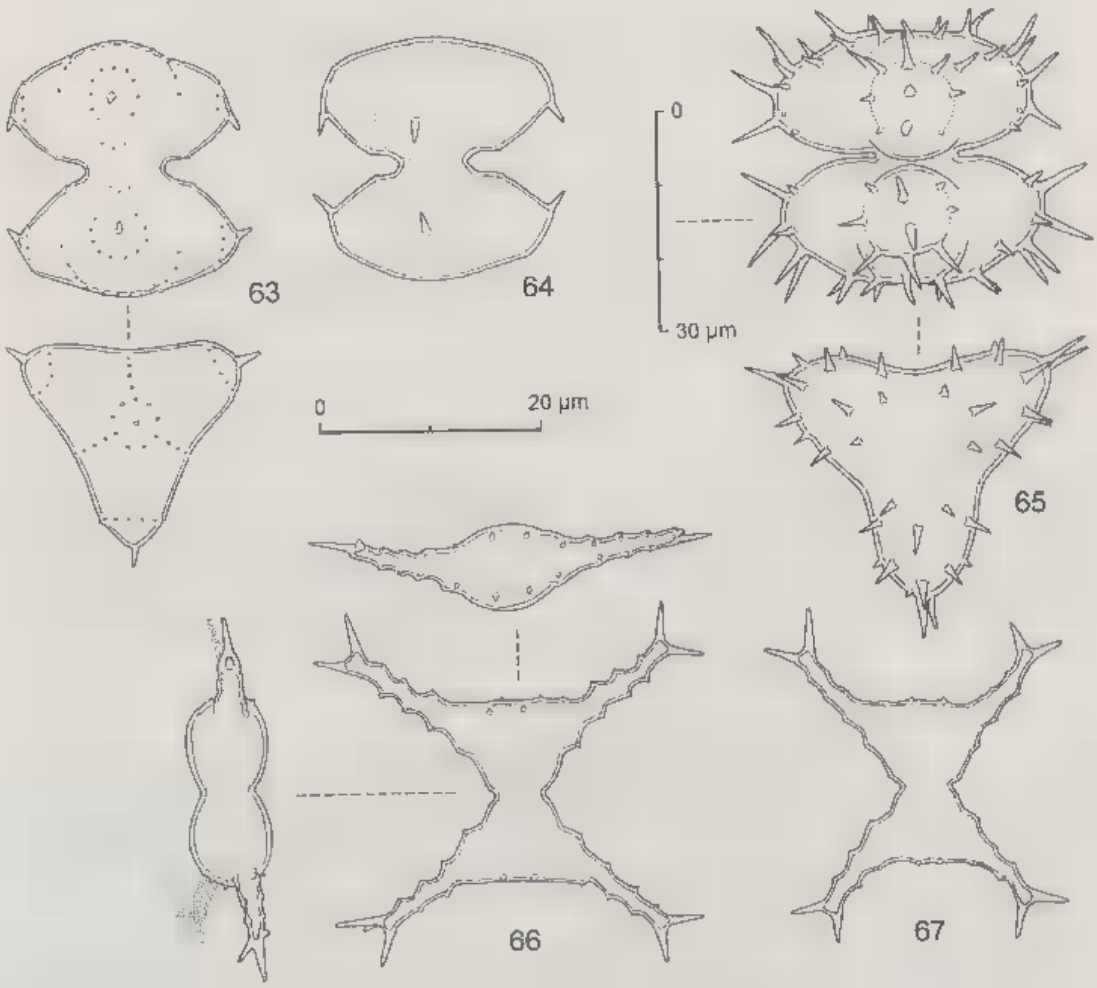
Figs 13-21. Figs 13-15. *Cosmarium jaoi*. Fig. 16. *C. haynaldii*. Figs 17-18. *C. limnophilum*. Figs 19-21. *C. sexnotatum* var. *bipunctatum*.



Figs 22-40. Figs 22-23. *Cosmarium berryense*. Figs 24-25. *C. lutetianum*. Figs 26-27. *C. dilatatum*. Fig. 28. *C.* sp. Figs 29-40. *C. angulosum* var. *concinnum*.



Figs 41-62. Figs 41-44. *Cosmarium asymmetricum*. Figs 45-52. *C. pseudowembarense*. Figs 53-55. *C. boitierense* var. *boitierense*. Figs 56-62. *C. boitierense* var. *inambitiosum*.



Figs 63-67. Figs 63-64. *Staurodesmus reginae*. Fig. 65. *Staurastrum gladiosum* var. *delicatulum*. Figs 66-67. *S. bloklandiae*.

Source : MNHN, Paris

BATRACHOSPERMACEAE (RHODOPHYTA) IN FRANCE: 200 YEARS OF STUDY

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ABSTRACT — Documentation of the Batrachospermaceae, a family of freshwater red algae, began in France in 1797. Much of the nomenclature in this family is based on the excellent herbarium specimens of J.-B.G.M. Bory de St-Vincent (1778-1846) and S. Sirodot (1825-1903). The recent morphometric-based system of R.G. Sheath, M.L. Vis and K.M. Cole has rationalized the multitude of names associated with these specimens. The Batrachospermaceae holdings of PC are here tabulated and cross-referenced to this system, and collections made by the author in 1992 are used to test the species concepts. Further collecting in France is recommended to assess environmental change and rarity, and to improve our concepts of taxa and taxonomic characters in the Batrachospermaceae.

RÉSUMÉ — La documentation sur les Batrachospermaceés, une famille d'algues rouges d'eau douce, a commencé en France en 1797. La nomenclature de cette famille est, en grande partie, basée sur les excellents échantillons d'herbier de J.-B.G.M. Bory de St-Vincent (1778-1846) and S. Sirodot (1825-1903). Le nouveau système de R.B. Sheath, M.L. Vis et K.M. Cole, basé sur la morphométrie, a rationalisé la multitude des noms qui leur ont déja été attribués. Un état des échantillons de *Batrachospermum* conservés à PC est dressé, sous la forme d'un tableau dans lequel ceux-ci sont rapportés à ce système. Les échantillons récoltés en France en 1992 par l'auteur lui-même sont analysés pour vérifier les concepts d'espèces. De nouvelles récoltes en France sont souhaitables pour estimer les modifications de l'environment, pour évaluer la rareté de certains taxons et pour améliorer nos concepts taxonomiques ainsi que les caractères sur lesquels ils reposent chez les Batrachospermacées.

KEY WORDS: *Batrachospermum*, freshwater red algae, history, nomenclature, taxonomy, systematics

INTRODUCTION

The purpose of this paper is three-fold: firstly, to provide a brief overview of the documentation of France's Batrachospermacean flora; secondly, to test the taxonomic system of Sheath and colleagues using contemporary collections from France; and thirdly, to draw some general conclusions about the abundance of species in France. In so doing,

I wish to honour Professor Pierre Bourrelly, whose books inspired me as a student to study freshwater algae, and since then have been invaluable companions in my studies.

HISTORICAL BACKGROUND

1797-1823: At the age of eighteen and in the fourth year of the First Republic, Jean Baptise Geneviève Marcellin Bory de Saint-Vincent (1778-1846) submitted a paper to the Société d'Histoire Naturelle de Bordeaux on the Linnaean genera *Conferva*, *Byssus* and *Phytoconis*. In that paper (1797), Bory described the species of these genera reported from the department of Gironde (Bory was Conservateur des Collections in the Société). Based on his own observations, he provided the first detailed description of *Conferva gelatinosa*. This species, he noted (on p. 38), was perhaps distinct enough to be separated from *Conferva*. In fact in Germany, in the same year, Albrecht W. Roth (1797) established the genus *Batrachospermum* to include *Chara gelatinosa* (L.) Roth [syn. *Conferva gelatinosa* L. (in error by Roth as "*Conferva nodosa* L.")] and *Chara batrachosperma* Weiss. Bory also recognized several variétés of this taxon, two of them growing in Gironde and matching Dillenius's (1741) *Conferva...major et fusca* and *Conferva...minor et viridis* (both = *Batrachospermum gelatinosum sensu* Vis *et al.*, 1995).

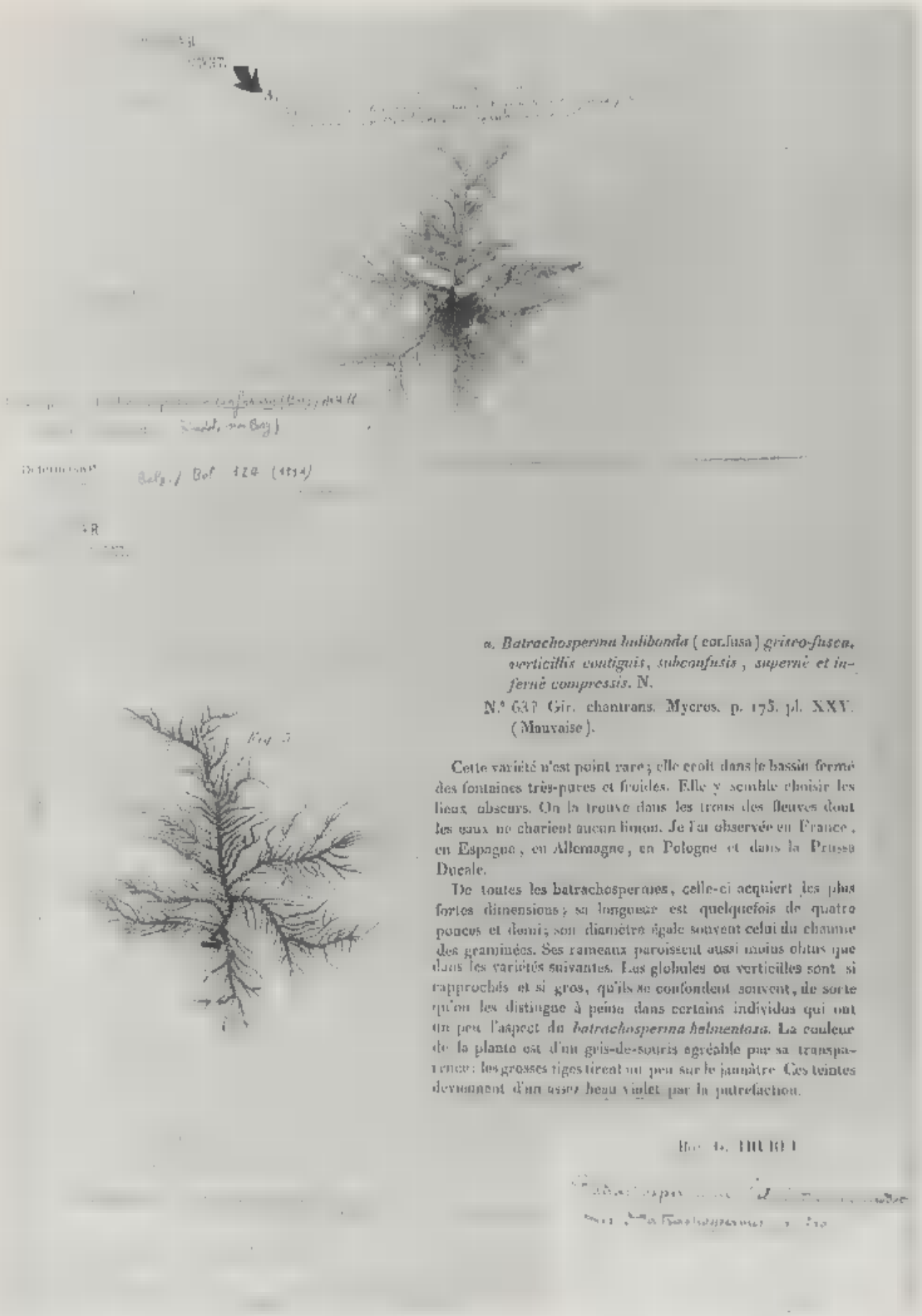
Bory was fascinated by freshwater red algae. While serving in Napoleon's army, he collected throughout Europe, including in 1802 the department of Ille-et-Vilaine (destined to become the geographical heart of *Batrachospermum* taxonomy upon publication of Simon Sirodot's monograph in 1884).

In 1808, Bory published three papers on the Batrachospermales. The first paper (1808a) concerned the genus *Thorea*. In his second (Bory 1808b), Bory includes among the six species of *Lemanea*, *L. sertularina*, *L. dillenii* and *L. batrachosperma*, all "setaceous" species of *Batrachospermum*. Collections were provided by compatriots such as D.S. Léman, J. Thore and J.P.R. Draparnaud. In his papers, as in his herbarium, new species are designated with a capital "N".

Bory's (1808c) third paper concerned the genus *Batrachosperma*. He includes the species *B. bambusina*, *B. helmintosa*, *B. ludibonda* (incl. "varieties" *confusa*, *aequinoxialis* from Réunion, *moniliforma*, *pulcherrima*, *cæ[æ]rulescens*, *viridis*, *stagnalis*), *B. turfosa*, *B. keratophyta* and *B. tristis* (= *Draparnaldia*; incl. "varieties" *chlora* and *colorata*).

In 1823, Bory transferred the three species of *Lemanea* mentioned above to *Batrachosperma* (the latter becoming *B. tenuissima*). An additional species, *B. hybrida*, is described, and *cærulescens* is raised to species level. Bory's fine herbarium (e.g. Fig. 1) was purchased by Gustav A. Thuret and now forms part of the collection at PC.

1867-1884: Simon Sirodot (1825-1903), school teacher, botanist and finally academic, discovered phycology relatively late in life. While Professor of Botany and Zoology at the Université de Rennes in the 1860s, his research assistant and bryologist, Jules Gallée, encouraged his interest in *Batrachospermum*. Sirodot's own collections were limited by how far he could travel by stagecoach (F. Magne, pers. comm.), but by 1873, when he described the sections *Moniliformia*, *Turfosa*, *Helminthosa* and *Virescentia*, he was intimately acquainted with the *Batrachospermum* flora of France. His own collections from north-western France were supplemented by the rich herbarium legacy of Bory and his compatriots. Sirodot's splendid monograph of 1884 is a fitting memorial to what became his life's work.



α. *Batrachosperma ludibonda* (confusa) *griseo-fusca, verticillis contiguis, subconfusis, superne et inferne compressis.* N.

N.° 637 Gir. chantrans. Mycros. p. 175. pl. XXV. (Mauvaise).

Cette variété n'est point rare; elle croît dans le bassin fermé des fontaines très-pures et froides. Elle y semble choisir les lieux obscurs. On la trouve dans les trous des fleuves dont les eaux ne charient aucun limon. Je l'ai observée en France, en Espagne, en Allemagne, en Pologne et dans la Prusse Ducale.

De toutes les batrachospermes, celle-ci acquiert les plus fortes dimensions; sa longueur est quelquefois de quatre pouces et demi; son diamètre égale souvent celui du chaume des graminées. Ses rameaux paroissent aussi moins obtus que dans les variétés suivantes. Les globules ou verticilles sont si rapprochés et si gros, qu'ils se confondent souvent, de sorte qu'on les distingue à peine dans certains individus qui ont un peu l'aspect du *batrachosperma helmentosa*. La couleur de la plante est d'un gris-de-souris agréable par sa transparence: les grosses tiges tirent un peu sur le jaunâtre. Ces teintes deviennent d'un assez beau violet par la putréfaction.

Fig. 1. *Batrachosperma ludibonda [ludibunda] confusa* Bory: Environs de Fougères en Bretagne, France, an VIII [1799-1800] (PC, Herbier Thuret). Holotype of *B. confusum* (Bory) Hassall.

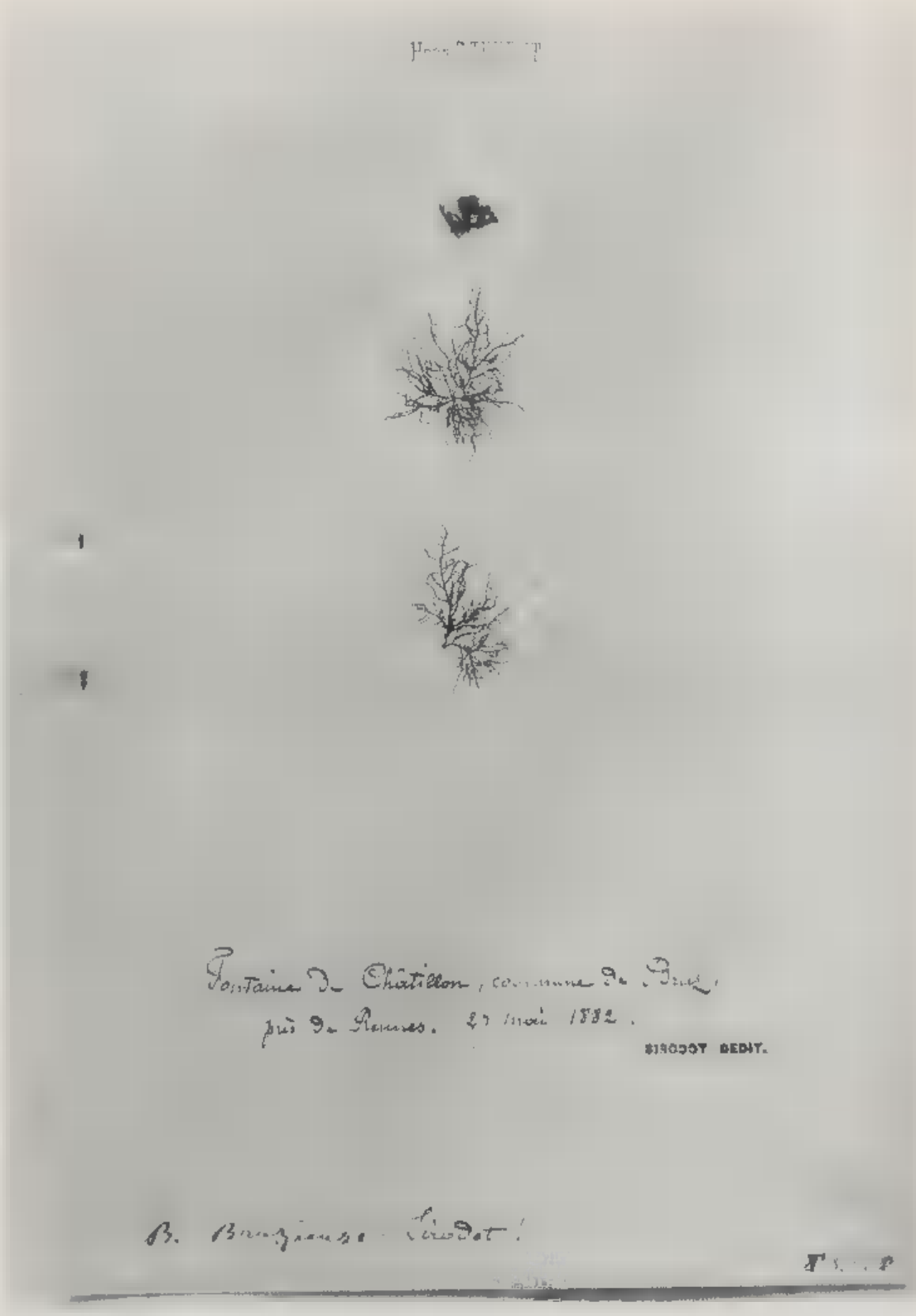


Fig. 2. *Batrachospermum bruziense* Sirodot: Fontaine de Châtillon, commune de Bruz, près de Rennes, 27 Mai 1882 (PC, Herbier Thuret). Middle specimen designated lectotype in Sheath *et al.* (1994a).

Sirodot made a number of nomenclatural errors (e.g. creating later synonyms and homonyms, and altering the ending of sectional names) but his monograph remains the only Flora of *Batrachospermum* for France. Hamel (1925) provides essentially a condensed version of Sirodot (1884) with some additional collections. Most of Sirodot's herbarium is housed at PC, and all specimens are adequately labelled and preserved (e.g. Fig. 2).

The twentieth century: Sirodot's species concepts were refined by Kylin (1912) and then more substantially challenged by Israelson (1942). Skuja added considerably to our knowledge of the family Batrachospermaceae (e.g. Skuja, 1931, 1944) but failed to complete his proposed world monograph. In the last few decades, beginning with Mori (1975), there has been a major reassessment of species concepts and a reexamination of types (e.g. Compère, 1991; Kumano, 1990; Necchi, 1990; Vis *et al.*, 1995). The recent revisionary work of R.G. Sheath, M.L. Vis and K.M. Cole (Sheath *et al.*, 1993, 1994a, 1994b, 1994c; Sheath & Vis, 1995; Vis *et al.*, 1995) used morphometric characters to rationalize (and greatly simplify) the taxonomy within the family, and particularly within *Batrachospermum* (Table 1).

Table 1. Nomenclatural and taxonomic changes in *Batrachospermum*

CURRENT NAME[1]	NAMES USED BY BORY & SIRODOT, 1808-1884	SPECIMENS FROM FRANCE HELD IN PC[2]
Section *Batrachospermum*[3]	**Sections *Moniliformes* & *Helminthoides***	
anatinum Sirodot	*anatinum* Sirodot *ectocarpum* Sirodot	31
arcuatum Kylin	—	17
boryanum Sirodot	*boryanum* Sirodot	11
confusum (Bory) Hassall	*crouanianum* Sirodot *helminthosum* Sirodot *non* Bory *ludibonda confusa* Bory	37
gelatinosum (L.) DC.	*corbula* Sirodot *decaisneanum* Sirodot *densum* Sirodot *godronianum* Sirodot *hybrida* Bory *ludibonda coerulescens* Bory *ludibonda pulcherrima* Bory *ludibonda stagnalis* Bory *moniliforme chlorosum* Sirodot *moniliforme helminthoideum* Sirodot *moniliforme rubescens* Sirodot *moniliforme scopula* Sirodot *moniliforme typicum* Sirodot *pygmaeum* Sirodot *pyramidale* Sirodot *radians* Sirodot *reginense* Sirodot	290
skujae Geitler	*sporulans* Sirodot	7
Section *Hybrida*	**Section *Hybrida***	
virgato-decaisneanum Sirodot	*virgato-decaisneanum* Sirodot	3

Section *Turfosa*	**Section *Turficoles***	
turfosum Bory	*keratophytum* Bory *turfosum* Bory *vagum* (Roth) C. Agardh *vagum keratophytum* (Bory) Sirodot *vagum suevorum* (Kütz. *nom. illeg.*) Sirodot	59
vogesiacum T.G. Schultz *ex* Skuja	*vagum flagelliforme* Sirodot	6
Section *Virescentia* (& *Setacea*)	**Sections *Verts* ■ *Setaces***	
atrum (Huds.) Harv.	*gallaei* Sirodot *dillenii* (Bory) Bory *tennuissimum* Bory *sertularinum* (Bory) Bory	93
elegans Sirodot	*coerulescens* Sirodot *elegans* Sirodot	21
helminthosum Bory *non* Sirodot	*bruziense* Sirodot *graibussoniense* Sirodot *helminthosum* Bory *non* Sirodot *testale* Sirodot *virgatum* Sirodot *viride* Sirodot	23
Names of doubtful application		
?*vagum* [Skuja *in sched.*]	*vagum* var. *affine* (Kütz.) Sirodot	2
?*vagum*	*vagum* var. *refractum* Sirodot	?0
?*vagum*	*vagum* var. *vulgare* Sirodot	?0
ascios [axios] Skuja *in sched.*	—	1
ectocarpoideum Skuja *in sched.*	—	2
myurus DC.	—	2
pulvinatum Bonhomme	—	1

[1] The "current name" is taken from the series of papers by Sheath and co-workers (Müller *et al.*, 1997; Sheath *et al.*, 1993, 1994a, 1994b, 1994c; Sheath & Vis, 1995; Vis *et al.*, 1995).

[2] The figures are approximate and some mistakes will have been made through misreading labels and ignoring duplication among "subherbaria". However the numbers reflect the relative frequency of collections in PC and (hopefully) to some extent the frequency of taxa in the field. Collections are housed in "PC Herbier de France", "PC Herbier Thuret", "PC Herbier Montagne" or "Reliquae Sirodotianae". The determinations are those used in the herbarium (many by Heinrichs Skuja) and none have been confirmed.

[3] The sections *Aristata*, *Contorta*, *Nothocladus*, *Sirodotia* and *Tuomeya* are not represented in France.

In France, the only major collections made this century were those included in the herbarium of E. Chemin (donated to PC), mostly by E. Cheuiy in the 1930s. Sixty seven specimens of *Batrachospermum* are housed in the herbarium of the Université de Rennes, including material labelled "Reliquae Sirodotianae" in the "Fonds Gallée". The collection was curated by Francis Magne between 1965 and 1971, when he taught at the university and studied the life history and development of Lemaneaceae from local

populations. All specimens collected by Sirodot and identified to species were transferred to PC.

Prior to Magne arriving in Rennes, Heinrichs Skuja had attempted to recollect from some of Sirodot's localities. Unfortunately the landscape had become much degraded, and Skuja was unable to find any *Batrachospermum* in the region (F. Magne, pers. comm.).

RECENT OBSERVATIONS

As part of a trip to France in 1992 to examine collections of Batrachospermaceae in PC, I too revisited some of Sirodot's collection sites near Rennes. Fortunately, I was able to find a few extant populations of *Batrachospermum*. I present these collections, and one from the Dordogne River valley in southern France, both as a test of the Sheath-Vis-Cole system (see above), as well as to stimulate further collecting in France to assess current day distributions.

Batrachospermum helminthosum Bory, *Ann. Mus. Hist. Nat.* 12: 316 (1808), *non* Sirodot (1884).

Specimen examined: Stream flowing into St-Malo-de-Beignon, Beignon-Launay Road, Paimpont region, 40 km SW of Rennes, route D124, 56-Morbihan, 2.x.1992, *Entwisle 2165* (MEL, PC).

Only young thalli were found, among "*Chantransia*" tufts in a heavily shaded creek. The carposporophytes are large and centrally inserted; the carpogonia symmetrically attached to the subtending cell and *ca* 45 µm long; the carpogonial branches straight, modified and *ca* 2 cells long; and the trichogynes pedicellate, cylindrical, and without knobs or branches. This combination of features matches *B. helminthosum sensu* Sheath *et al.* (1994a).

Distribution: Sirodot collected *B. helminthosum*, and current synonyms (Table 1), from many streams within a 50 km radius of Rennes. Although now reported from most continents (but not Australia) it has not been widely reported in France outside Brittany. The most recent collection in PC was also from Paimpont, in 1969.

Batrachospermum virgato-decaisneanum Sirodot, *Batrachospermes* 290 (1884).

Specimen examined: Le Meu River, Moulin de Dompierre, Trémorel, *ca* 50 km W of Rennes, route N164, 22-Côtes du Nord, 2.x.1992, *Entwisle 2167* (MEL).

A small, bright green fragment only was found at this site. With carpogonia *ca* 22 µm long and asymmetrically attached; trichogynes pedicellate and sessile; and the carpogonial branch 3-celled and modified, this collection is clearly referable to *B. virgato-decaisneanum*.

Distribution: This is the first record of *B. virgato-decaisneanum* from France since the collections of Sirodot in 1883. Sirodot reported it only twice, from near Montfort, 20 km W of Rennes. My collection was a fragment only and further searching for this species is warranted. *Batrachospermum virgato-decaisneanum* has been reported from elsewhere in Europe and also North and South America, Japan, Australia and New Zealand (Sheath & Vis, 1995; Entwisle, 1993). Although widespread, it is apparently uncommon worldwide.

Batrachospermum confusum (Bory) Hassall, *Hist. Br. Freshw. Algae* 1: 105 (1845).

Specimens examined: Stream from Roc Trévezel into Réservoir de St Michel, Botmeur-la Feuillée Road, first river crossing E of Botmeur township, *ca* 10 km WNW of Huelgoat, route D42, 29-Finistère, 30.ix.1992, *Entwisle 2157* (MEL, PC); Le Meu River, below Forêt de la Hardouinais, near Trémorel, *ca* 8 km W of St-Méen-le-Grand, route N-164, 22-Côtes du Nord, 2.x.1992, *Entwisle 2168* (MEL, PC).

The grey-olive thalli were attached to rocks in flowing water. Carposporophytes are numerous, small, and *ca* 14 cells from axis; spermatangia are borne on involucral bracts; and rhizoidal filament cells are swollen in mature axes. These characters define *B. confusum sensu* Vis *et al.* (1995).

Distribution: Species now included under *B. confusum* were reported commonly by Sirodot from the region around Rennes, extending NW to Saint-Pol-de-Léon. The most recent collections in PC were made prior to World War II.

Batrachospermum gelatinosum (L.) DC., *Bull. Sci. Soc. Philomat. Paris* 3 (51): 21 (1801).

Specimen examined (1): Small stream flowing out below Meyrals Chateau, St Cyprien-Meyrals-Sarlat Road, *ca* 2 km from St Cyprien, route D25, 24-Dordogne, 21.x.1992, *Entwisle 2176* (MEL, PC).

The thalli, growing with *Vaucheria* in a small pool, were grey, rubbery and *Chaetophora*-like in texture. With carposporophytes small and scattered through the whorls; carpogonia subtended by unmodified branches; rhizoidal filaments loose and tangled but the cells remaining cylindrical; and the population apparently monoecious (all individuals examined bore carpogonia, and spermatangia were observed attached to trichogynes), the collection is referable to *B. gelatinosum sensu* Vis *et al.* (1995).

Specimen examined (2): Le Buisson, *ca* 2 km SW of St-Malon-sur-Mel, Paimpont Forêt, 35-Ille-et-Vilaine, 2.x.1992, *Entwisle 2164* (MEL, PC).

This collection of small thalli from a slow-flowing stream was difficult to identify due to the rarity of key diagnostic features in a limited amount of fertile, new growth. Diagnostic features were as above, but the rhizoidal filaments were sometimes undulate or with an irregular surface (but the cells never inflated). Once again the only spermatangia observed were attached to trichogynes but all specimens observed bore carpogonia, so the population is assumed to be monoecious.

In older parts of the thalli, secondary fascicles were profuse and as long as primary fascicles, resulting in elongate, cylindrical whorls. This gross morphology is apparently unusual for European *B. gelatinosum* (see e.g. illustrations in Vis *et al.*, 1995), but not unexpected for "overmature" individuals of any species of *Batrachospermum*.

No unfertilized trichogynes were observed and most fertile branches were bearing carposporangia, even those with limited apparent gonimoblast development. These carposporangia were 6-8 µm long and globose to obovoid, not all that different in size and shape to the spermatangia attached to trichogynes. However, they seemed to be consistently bigger than the spermatangia and were always associated with fertilized trichogynes (but note that no unfertilized trichogynes were observed). Furthermore, if they were spermatangia, the collection would be allied with *B. pulchrum* which unlike *Entwisle 2164* has well-curled fascicles (Vis *et al.*, 1995). The most pragmatic approach is to refer this collection to *B. gelatinosum*.

Distribution: Although widespread throughout the world, *B. gelatinosum* has over the last century become less common on the Eurasian continent, particularly near large cities (Geissler, 1991; Usachjova, 1995).

DISCUSSION

At 13 species (using current concepts), the French Batrachospermaceae flora in not particularly rich. Recent field and herbarium studies in Australia, suggest that there are *ca* 25 species in that country. While Australia covers a broader range of climate zones than France, the greatest species diversity is found in the southern temperate zones. Of more interest than simple species tallies is the fact that there are few species shared by both countries: that is, there are many endemics in Australia. However, *B. gelatinosum* and *B. atrum* seem to be the most widespread and common of species in both countries (although current concepts may neglect important variability). Most species reported from France are also found in other northern hemisphere regions such as North America, a region which boasts a total of some 30 species. Nevertheless, the flora of France is of immense historical and nomenclatural interest. Phylogenetic relationships are not yet resolved in Batrachospermaceae so further biogeographic analysis is premature.

Post World War II collections of Batrachospermaceae from France are rare in PC, and apart from a brief flourish in the 1930s, there are very few collections from this century. However, the French Batrachospermaceae flora must be one of the best documented of any "plant" group for the nineteenth century. In particular, the flora of the Rennes region is extremely well vouchered. As one would expect, recent intensive sampling near large cities in Europe *sensu lato* (e.g. Berlin, Geissler, 1991; Moscow, Usachjova, 1995) has shown Batrachospermaceae to be extinct or threatened with extinction. Even more widely in Europe, the family appears to be rare or at least rarely sighted (e.g. Freidrich *et al.* 1984). As Freidrich *et al.* (1984) note, systematic collecting is needed to assess the current distribution and abundance of Batrachospermaceae in Europe. Systematic collecting throughout France, particularly concentrating of sites visited by Sirodot, would form the basis of a very useful comparative study. Changes in habitats over the last century or so should be reflected in the presence or absence of Batrachospermaceae species.

The system of Sheath and co-workers accommodated all collections documented here: deviations can be explained by the poor quality of the specimen. It is possible that new taxa will be discovered in France, particularly outside Brittany. It is also possible that further analysis of individual characters may show that the taxa defined by dissimilarity of multiple morphometric characters obscure phylogenetically and/or phenetically distinct entities. The character of monoecy vs dioecy, e.g., requires further study. Vis *et al.* (1995) include two "species pairs" that differ only (or almost only) in this regard. This character, with the two states monoecious and dioecious, has no *intrinsic* value taxonomically or phylogenetically (despite the impassioned pleas of e.g. Proctor, 1975). In a study in progress, we find this simplistic scoring of the character inadequate, and its suitability as a taxonomic character suspect in many instances. Devaluing this character would result initially in fewer species being recognized, but an assessment of all vegetative and reproductive features may result in the discovery of more informative characters. Also, the distribution of female and male gametangia may in fact provide a number of more complex characters that can be better used to interpret phylogeny. To improve our knowledge of diversity and relationships, we need to analyse the development, distribution and evolution of characters.

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Source : MNHN, Paris

CHROOCOCCIDIOPSIS BOURRELLYANA NOV. SP. (CYANOPHYTA, XENOCOCCACEAE)

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ABSTRACT — A new species of the genus *Chroococcidiopsis* Geitler, *C. bourrellyana*, is described from brackish waters in the lower part of the Sine and Saloum rivers in Senegal. The new species differs from other taxa described in the genus chiefly by larger dimensions of the cells and sporangia and by the great numbers of nanocytes formed in mature sporangia. A key allows the diffenrentiation of the new species from the other species of the genus *Chroococcidiopsis*.

RÉSUMÉ — Une nouvelle espèce du genre *Chroococcidiopsis* Geitler, *C. bourrellyana*, est décrite des eaux saumâtres du cours inférieur des rivières Siné et Saloum, dans l'ouest du Sénégal. La nouvelle espèce se distingue des autres taxons décrits dans ce genre par les dimensions plus importantes des cellules et des sporocystes, ainsi que par le grand nombre de nanocytes formés dans les sporocystes adultes. Une clé de détermination permet de situer la nouvelle espèce au sein du genre.

KEY WORDS: *Chroococcidiopsis*, Cyanophyta, morphology, new species, Senegal, taxonomy.

INTRODUCTION

Lors d'une excursion botanique organisée avec des collègues du Jardin Botanique National de Belgique, j'ai eu l'occasion de récolter des algues dans divers milieux aquatiques d'eau douce ou d'eau saumâtre à travers le Sénégal, depuis le fleuve Sénégal et le lac de Guiers au nord, jusqu'à la Casamance, au sud (Compère, 1991). Dans deux des échantillons examinés se trouve en abondance une algue bleue coccoïde qui se rapproche du genre *Chroococcidiopsis* Geitler par son mode de reproduction, mais qui se distingue des autres espèces décrites dans ce genre par les grandes dimensions de ses cellules et les nombreux nanocytes formés dans ses sporocystes.

MATÉRIEL ET MÉTHODES

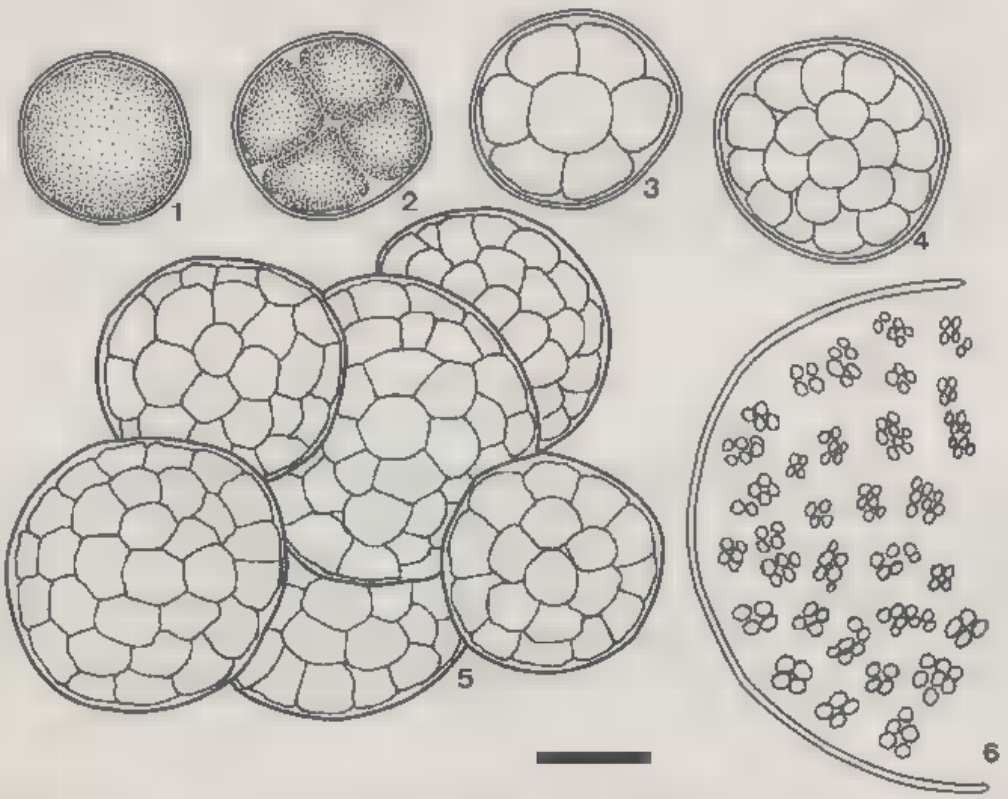
Le matériel a été récolté en novembre 1984, dans le cours inférieur des rivières Siné et Saloum, là où les cours d'eau sont encore sous l'influence des marées et où l'eau est nettement saumâtre. Les algues ont été récoltées au moyen d'un filet à plancton (vide de

maille 25 μm) et fixées sur place par l'addition de formol dans l'eau de récolte jusqu'à obtention d'une solution à environ 4 %. Ces échantillons sont déposés dans l'herbier cryptogamique du Jardin Botanique National de Belgique (BR).

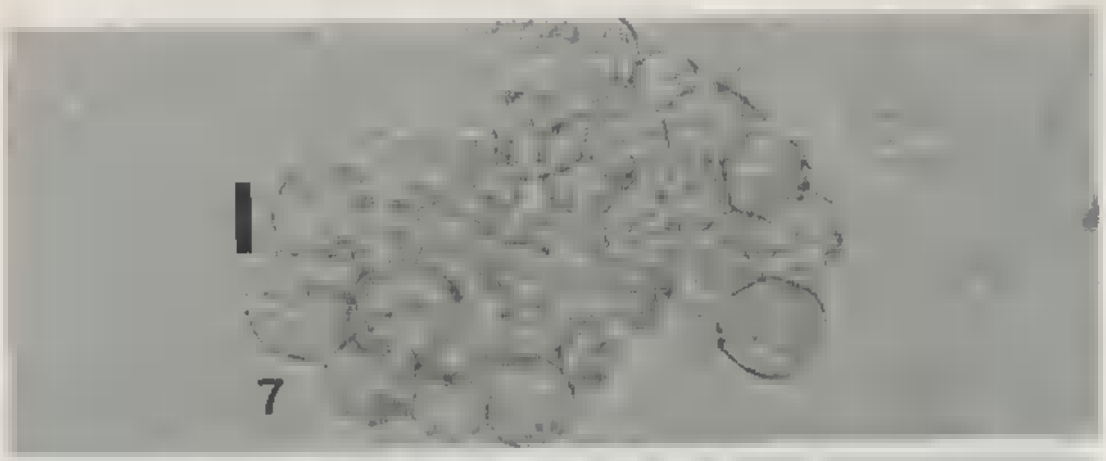
Les algues ont été examinées au moyen d'un microscope optique Zeiss Universal, équipé pour le fond clair, le contraste de phase et le contraste interférentiel et photographiées sur un film négatif noir et blanc Agfapan APX 100.

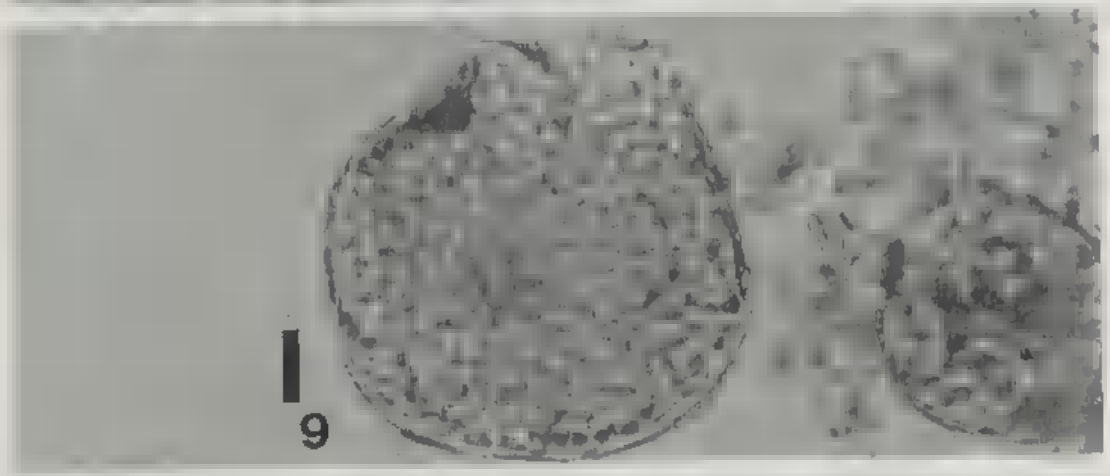
OBSERVATIONS

L'algue observée est formée de cellules isolées (Fig. 1) ou réunies en petits groupes irréguliers (Fig. 5) ; ces cellules sont globuleuses ou subglobuleuses et atteignent un diamètre de 10-16 μm. Leur paroi externe est ferme et parfois assez épaisse.



Figs 1-6. *Chroococcidiopsis bourrellyana* Compère — Fig. 1. Cellule isolée. Figs 2-4. Jeunes sporocystes (cellules productrices de nanocytes) à divers stades de développement. Fig. 5. Groupe de sporocystes. Fig. 6. Sporocyste adulte, avec nanocytes (endospores) en petits groupes correspondant aux divisions ultimes du protoplasme. Le trait d'échelle correspond à 10 μm.





Figs 7-9. *Chroococcidiopsis bourrellyana* Compère. Fig. 7. Groupe de jeunes sporocystes aux premiers stades de division. Fig. 8. Groupe de sporocystes à un stade plus avancé. Fig. 9. Sporange mûr, émettant des nanocytes. Les traits d'échelle correspondent à 10 μm.

Figs 10-11. *Chroococcidiopsis bourrellyana* Compère. Fig. 10. Jeune sporocyste en cours de division. Fig. 11. Nanocytes (endospores) formés en groupes correspondant aux divisions ultimes du protoplasme. Les traits d'échelle correspondent à 10 μm.

Elles se transforment rapidement en " sporocystes " (= cellules produisant des nanocytes, Figs 2-8, 10) et leur contenu subit des divisions successives pour former de nombreuses cellules-filles (Figs 6, 9, 11). Celles-ci ont été le plus souvent nommées " endospores " (Geitler, 1933, 1942 ; Bourrelly, 1970 ; Komárek & Hindák, 1975) et parfois " baeocytes " (Waterbury & Stanier, 1978) ; ces termes ont été critiqués par Komárek & Anagnostidis (1986 : 168) qui préfèrent utiliser le terme " nanocytes ". C'est ce dernier terme qui sera utilisé ici. Faute d'un terme plus approprié nous continuerons cependant à nommer " sporocystes " les cellules productrices de nanocytes (cf. Dor *et al.*, 1991). Pour autant qu'il soit possible d'en juger sur du matériel fixé, le mode de division de l'espèce sénégalaise semble correspondre au type D de Friedmann (1961) et des divisions correspondant à des cellules-mères de deuxième, troisième ou quatrième ordre sont repérables sur des sporocystes en cours de développement (Figs 7, 8, 10). Les premières divisions s'observent déjà sur des cellules de 12 µm de diamètre (Fig. 7) ; les sporocystes croissent au fur et à mesure des divisions pour atteindre un diamètre de 40 à 70 µm au moment de l'expulsion des nanocytes par déchirure de la paroi (Fig. 9).

Dans le système proposé par Komárek & Anagnostidis (1986), ces caractères correspondent à ceux du genre *Chroococcidiopsis* Geitler (1933) ; parmi les espèces retenues dans ce genre par Komárek & Hindák (1975), elle se rapproche de *C. kashaii* Friedmann (1961) par le mode de division et par le grand nombre de nanocytes formés, mais cette espèce à des cellules plus petites, ne dépassant pas 6,5 µm de diamètre et ses sporocystes ne dépassent pas 31,5 µm de diamètre. De plus son écologie est bien différente puisqu'il s'agit d'une algue aérophile, colonisant des parois de grottes en Israël. La grande dimension des sporocystes distingue aussi le matériel sénégalais de toutes les espèces décrites dans le genre. Dès lors il paraît légitime de considérer ce matériel comme représentant une nouvelle espèce que je suis heureux de pouvoir dédier à la mémoire du Professeur Bourrelly qui a guidé mes premiers pas d'algologue débutant et qui m'a toujours fort aimablement accueilli lors de mes visites au Laboratoire de Cryptogamie du Muséum d'Histoire Naturelle de Paris.

***Chroococcidiopsis bourrellyana* Compère, sp. nov. — Figs 1-11.**

Cellulae globosae vel subglobosae, solitariae vel in familiis irregulatim conglomeratae, contentu homogeneo, griseo-aerugineo, 9-16 µm in diametro. Paries cellulae firmus, achrous. " Sporangia " ad 60 µm in diametro. Nanocytae irregulariter globosae, subangulares, 2-3 µm in diametro, succesivis divisionibus formatae.
Holotypus : Coll. P. Compère 4343, Nov. 1984 (BR).
Locus typicus : Fatik (Senegal), 16°23'W — 14°20'N, *in plancto fluminis Siné*.

Dans la récolte type, la nouvelle espèce était accompagnée d'autres Cyanophycées dont les plus abondantes étaient *Gomphosphaeria aponina* Kütz. et *Johannesbaptistia pellucida* (Dickie) Taylor & Drouet et d'un cortège de diatomées caractéristiques des eaux saumâtres comme *Amphora coffeiformis* (Ag.) Kütz., *A. proteus* Greg., *Brachysira aponina* Kütz., *Mastogloia aquilegiae* Grun. *ex* A. Schmidt, *M. sirbonensis* Ehrlich, *M. smithii* var. *heteroloculata* Ehrlich, *Pleurosigma delicatulum* W. Sm., etc. Elle a été retrouvée dans le plancton des eaux claires du cours inférieur du fleuve Saloum, en amont de Kaolack (16°02'W — 14°09'N, coll. P. Compère 4345) accompagnée des mêmes Cyanophycées et d'autres diatomées des eaux saumâtres comme *Actinocyclus normanii* (Greg. *ex* Grev.) Hust. *ex* VanLandingham, *Gyrosigma balticum* (Ehrenb.) Rabenh., *Mastogloia braunii*

Grun., *Navicula mollis* (W. Sm.) Cleve, *N. duerrenbergiana* Hust., *Nitzschia compressa* var. *balatonis* (Grun.) Lange-Bertalot, *Surirella striatula* Turpin, *Thalassiosira eccentrica* var. *fasciculata* (Hust.) Nizamuddin, etc. Dans ces deux récoltes *Chroococcidiopsis bourrellyana* était bien abondant, le plus souvent sous la forme de sporocystes à divers stades de leur développement.

DISCUSSION

Le genre *Chroococcidiopsis* a été établi par Geitler (1933), avec une seule espèce, *C. thermalis* Geitler, des sources chaudes de Kadjaj à Sumatra. Il est placé dans la famille des Cyanidiaceae Geitler, à côté d'un autre nouveau genre, *Cyanidium* Geitler, dont il diffère essentiellement par le plus grand nombre d'endospores (= nanocytes). Dans sa révision des Cyanophyceae pour la 2e édition des " Naturliche Pflanzenfamilien ", Geitler (1942) maintient la même classification mais émet certains doutes quand à l'appartenance de *Cyanidium* aux Cyanophyceae. Dès lors, au cas où il serait avéré que *Cyanidium* n'est pas une Cyanophyceae, il propose de donner à la famille le nom de Chroococcidiopsidaceae. Il s'agit là d'un nom provisoire au sens du Code international de la nomenclature botanique (CINB, art. 34.1 : Greuter *et al.* 1994) ; un tel nom n'est pas validement publié. Drouet & Daily (1956) considèrent le type de *Cyanidium* comme appartenant au genre *Chlorella* Beijerinck (Chlorophyceae) et font de *Chroococcidiopsis thermalis* un synonyme de *Anacystis montana* (Lightf.) Drouet & Daily qu'ils classent dans les Chroococcaceae. Par contre, Bourrelly (1970) reconnaît la validité de *Chroococcidiopsis*, mais le classe dans la famille des Clastidiaceae Drouet & Daily où il diffère cependant des autres genres par l'absence de différenciation entre base et sommet. Ce caractère incite Komárek *et al.* (1975) à créer, au sein des Clastidiaceae, la sous-famille unigénérique des Chroococcidiopsidoideae, caractérisée par l'absence de polarisation des cellules. Enfin, dans leur nouvelle approche de la classification des Cyanophytes, Komárek & Anagnostidis (1986) incluent *Chroococcidiopsis* dans la famille des Xenococcaceae qui regroupe des genres dont les cellules non polarisées se reproduisent par divisions successives selon divers plans, pouvant conduire à la production de nanocytes. C'est cette dernière position qui est adoptée ici. Une révision des genres de cette famille et notamment de *Myxosarcina* Printz (1921), *Endospora* Gardner (1927) et *Asterocapsa* Chu (1952) serait pourtant nécessaire pour bien préciser les limites et le contenu de *Chroococcidiopsis* ; de même, il serait souhaitable de définir plus clairement le genre *Anacystis* Menegh., notamment par l'étude de son type nomenclatural (*A. marginata* Menegh.), afin de clarifier sa position au sein du système de Komárek & Anagnostidis (1986). On peut simplement signaler qu'*Endospora* est considéré comme synonyme d'*Anacystis* par Drouet & Daily (1956) et de *Myxosarcina* par Komárek & Anagnostidis (1986) tandis qu'*Asterocapsa*, traité comme synonyme du genre de Chlorophycée *Palmogloea* Kütz. par Drouet & Daily (1956) et omis par Komárek & Anagnostidis (1986) est maintenant considéré comme un bon genre de Cyanophycée par Komárek (1992, 1994). Ce genre présente toutefois des formes de résistance à parois épaisses et ornementées (" arthrospores ") qui n'ont pas été observées dans le matériel sénégalais.

À ce jour, une quinzaine d'espèces ont été reconnues dans le genre *Chroococcidiopsis*, le plus souvent de régions tropicales ou subtropicales. On pourrait y ajouter quelques espèces décrites dans le genre *Anacystis* Menegh. et caractérisées par des parois cellulaires épineuses ou granuleuses, souvent colorées, notamment *A. magnifica* Gardner, *A. pulchra* Gardner et *A. amplivesiculata* Gardner de Porto-Rico (Gardner, 1927) ou

encore *A. purpurea* Jao et *A. trochiscioides* Jao, de Chine (Jao, 1944) ; des incertitudes sur leur mode de reproduction empêche le transfert formel de ces espèces dans le genre *Chroococcidiopsis*, bien qu'elles soient reprises dans le tableau du genre présenté par Komárek & Hindák (1975). La petite clé ci-dessous permettra de situer la nouvelle espèce au sein du genre *Chroococcidiopsis*. Elle reprend la plupart des espèces classées dans ce genre mais, faute de données sur le nombre de nanocytes par sporocyste, il n'a pas été possible d'y inclure trois espèces récemment décrites de plusieurs localités israéliennes par Dor *et al.* (1991) : *C. supralittoralis* Dor *et al.*, *C. umbratilis* Dor *et al.* et *C. versatilis* Dor *et al.* ; les deux premières proviennent de milieux aérophiles et ont des sporocystes ne dépassant pas 20 µm de diamètre, ce qui les différencie nettement de l'espèce du Sénégal et la troisième s'en distingue par des cellules plus petites, ne dépassant pas 7 µm de diamètre.

Clé des espèces du genre *Chroococcidiopsis*

1. Paroi des cellules couvertes d'épines ou d'excroissances coniques 2
   Paroi cellulaire sans épines 3
2. Épines fines ; sporocystes à 24 nanocytes ..... *C. karnatakensis* Kamat
   Épines plus épaisses ; sporocystes à 64 nanocytes ..... *C. spinosa* Kamat
3. Nanocytes minuscules, de 0,5 µm de diam. ; sporocystes de 4 µm de diam. ; espèce marine ..... *C. fissurarum* (Erceg.) Komárek & Anagnost.
   Nanocytes de plus de 1 µm de diam. 4
4. Sporocystes ne contenant jamais plus de 32 nanocytes 5
   Sporocystes contenant souvent plus de 32 nanocytes 8
5. Sporocystes de moins de 10 µm de diam., à 4 nanocytes . *C. doonensis* R.B. Singh
   Sporocystes à 8-32 nanocytes 6
6. Sporocystes à 8-12-(16) nanocytes ; algue non liée aux eaux thermales 7
   Sporocystes renfermant jusque 32 nanocytes, atteignant 18 µm de diam. ; algue des eaux thermales ..... *C. thermalis* Geitler
7. Cellules sphériques de 5-7 µm de diam. ; eaux douces, en culture ..... *C. indica* Desikachary
   Cellules ellipsoidales, de 3, 8-6, 7-(7,5) × 3, 8-4, 5-(6,7) µm ; marin, épiphyte sur *Codium* ..... *C. codiicola* Beljakova
8. Cellules végétatives ne dépassant pas 7 µm de diam. ; algue aérophile et sciaphile de parois de grottes ; nanocytes nombreux ..... *C. kashaii* Friedmann
   Cellules végétatives de plus de 10 µm de diam. 9
9. Sporocystes atteignant 60 µm de diam. et renfermant de nombreux nanocytes ; cellules végétatives atteignant 16 µm de diam. ..... *C. bourrellyana* Compère
   Sporocystes ne dépassant pas 35 µm de diam. 10
10. Cellules végétatives de 10-13 µm de diam. ; sporocystes de 25-28 µm de diam., renfermant rarement plus de 64 nanocytes ..... *C. cubana* Komárek & Hindák
    Cellules végétatives de 14 µm de diam. ; sporocystes de 34 µm de diam, pouvant renfermer plus de 100 nanocytes ..... *C. mysorensis* Tiwari

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CHEMISTRY OF THE SHEATH OF THE CYANOBACTERIUM *LYNGBYA AESTUARII* LIEB.

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Dedicated to the memory of Professeur Pierre Bourrelly

ABSTRACT — The sheath of the cyanobacterium *Lyngbya aestuarii* has been shown to be a sulfated proteoglycan. The polypeptide comprises 12.9% of the sheath dry weight and sulfate esters account for 2.0%. Aspartic acid and alanine represent 32.5% of the polypeptide component. The carbohydrate moiety contains arabinose, galactose, glucose, mannose, rhamnose, xylose and the uronic acids, galacturonic and glucuronic acid. The dominant monosaccharide is glucose, averaging 18.0% of the dry weight. At least 13 different monosaccharide linkages have been identified. The data from these analyses suggest that this morphologically rigid sheath is a single sulfated proteoglycan. The ultraviolet shielding pigment scytonemin, while located in the sheath, is not an integral part of the sheath. Scytonemin is easily removed by solvent extraction and there are no significant differences in the composition of pigmented and unpigmented sheaths.

RÉSUMÉ — Cette étude montre que la gaine de la cyanobactérie *Lyngbya aestuarii* est un protéoglycane sulfaté. Le polypeptide représente 12,9% de la masse sèche de la gaine et les ester-sulfate 2,0%. Il est composé pour 32,5% d'acide aspartique et d'alanine. La fraction hydrocarbonée contient de l'arabinose, du galactose, du glucose, du mannose, du rhamnose, du xylose, de l'acide galacturonique et de l'acide glucuronique. L'ose dominant est le glucose qui représente en moyenne 18,0% de la masse sèche. Des liaisons entre, au moins, 13 oses différents ont été identifiées. Les données de ces analyses suggèrent que cette gaine morphologiquement rigide est constituée d'un seul protéoglycane. Le pigment protecteur contre les ultra-violets, la scytonémine, bien que située dans la gaine, n'en est pas partie intégrante. La scytonémine est aisément extraite à l'aide d'un solvant et il n'existe pas de différences significatives entre les compositions des gaines pigmentées et dépigmentées.

KEY WORDS: carbohydrates, Cyanobacteria, *Lyngbya*, marine microalgae, protein, sheath, scytonemin.

INTRODUCTION

Lyngbya aestuarii is an unbranched filamentous cyanobacterium belonging to Section III of the cyanobacteria complex according to the classification of Rippka, *et al* (1979). A filament of *L. aestuarii* is composed of a trichome of disc-shaped cells surrounded by a 1-3 µm thick firm fibrillar sheath (Lang, 1968). The trichome has the ability to move through the cylinder of the sheath, principally in response to light. Empty sheaths, lacking trichomes, retain their shape and are often observed in cultures. *L. aestuarii* typically grows as a felt-like mat of intertwined filaments in the intertidal zone of marginal marine and coastal hypersaline environments. Both morphology and growth habit promote trapping of sediment particles, which may also be bound to the sheath surface. Thus, net accretion and preservation of stromatolitic material may occur under favorable environmental conditions (Bauld, 1981).

Under field conditions the habitat of *L. aestuarii* mats receives intense solar radiation and sheaths at the surface of the mat become pigmented with scytonemin, a yellow-brown pigment, which is deposited in the sheath. The sheaths just below the surface layer remain unpigmented. The function of this pigmentation as an effective ultraviolet screen has been the focus of ■ series of investigations by Garcia-Pichel & Castenholz (1991) and Garcia-Pichel *et al.* (1992). The chemical structure of scytonemin has been determined by Proteau *et al.* (1993).

L. aestuarii bears a close resemblance to Precambrian microfossils with preserved sheath segments from the Early and Late Proterozoic, 2.3 10^8 to 8.5 10^8 years ago, (Schopf, 1968; Klein *et al.*, 1987). Attempts to artificially fossilize *Lyngbya* filaments resulted in artificial fossils which resembled their naturally occurring counterparts (Oehler & Schopf, 1971). Individual cells of the trichome were not preserved but the tubular structure of the sheath was organically preserved and clearly distinguishable. Bartley (1996) confirmed that the sheath of *L. aestuarii* was significantly more resistant to decomposition than the cells of the trichome.

Understanding the preservation properties of *Lyngbya* sheaths requires a knowledge of their chemistry. Since the sheath plays a role in UV protection and shows pigmentation in response to high irradiance, the possibility of chemical variation in the sheath must be considered, together with the question of whether or not scytonemin is an integral part of the sheath, possibly contributing to its structural integrity. This paper describes the chemistry of the sheath of *Lyngbya aestuarii* LM 8701. The data indicate that the sheath chemistry itself is not influenced by irradiance levels, and accumulated scytonemin remains chemically independent of the sheath structure.

METHODS

Organism and growth conditions. *Lyngbya aestuarii* Lieb. strain LM 8701 is an isolate collected from Shark Bay in Western Australia by J. Bauld. Axenic cultures were grown in modified Erdschreiber medium formulated as follows: 1 liter of artificial sea water constituted by dissolving 32 g of sea salts (Sigma Chemical Co.) in distilled water and bringing the volume to 1 liter. The following supplements were added: 200 mg

$NaNO_3$, 20 mg K_2HPO_4, 1 ml Fe-EDTA solution (4 g $FeSO_4$ $7H_2O$, 5 g Na_2EDTA and distilled water to 1 liter), 30 ml PII metal mix [1 g Na_2EDTA, 1.1 g H_3BO_3, 48 mg $FeCl_3$ $6H_2O$, 144 mg $MnCl_2$ $4H_2O$, 10.4 mg $ZnCl_2$, 4.3 mg $COCl_2$ $6H_2O$ and distilled water to 1liter (Provasoli *et al.*, 1957)], and 1 ml vitamin solution (500 mg thiamin, 10 mg vitamin B12, 20 mg biotin, 500 mg niacinamide, 100 mg p-aminobenzoic acid and distilled water to 1 liter). Cultures were grown at 25-30° C in 2.8 liter Fernbach flasks stoppered with foam plugs. Continuous irradiance of 15-30 μEm^{-2} sec^{-1} was provided to the cultures by a 15 watt cool-white fluorescent bulb. These culture conditions produced a felt-like mass of filaments characterized by colorless sheaths. Pigmentation of the sheath was induced by placing sections of the cultured mat, with medium, in glass petri dishes under continuous irradiance of 140 μEm^{-2} sec^{-1} produced by a bank of cool-white fluorescent bulbs suspended 10 cm above the culture.

Isolation of sheath. The aggregated mat of filaments of *L. aestuarii* was removed intact from the culture flasks or petri dishes and washed with distilled water. Portions of the mat were first frozen in liquid nitrogen then ground to a powder with dry ice in a pre-cooled electric coffee grinder. Sheath material, free of intact cells, was prepared by the method of Adhikary *et al.* (1986), modified for the high density of the sheath fragments. Aliquots of the powdered sample were suspended in a volume of distilled water equal to 10 × the original wet weight of cell mass and passed through a French pressure cell at 96 MPa. The crude sheath fraction was recovered from the ruptured filaments by low speed centrifugation (15 min at 3,500-5,000 g) in a swinging bucket rotor and washed 4 × with distilled water or until the supernatant was no longer green. The pellet containing the sheath was resuspended in 10 mM Tris-HCl buffer pH 8.0 and treated overnight at 42° C with egg-white lysozyme (EC 3.2.1.17) at an enzyme concentration of 20 mg ml^{-1} to facilitate removal of any residual cell wall from the sheath. The treated sheath fraction was recovered by low speed centrifugation and the pellet containing the sheath was washed 2 x with distilled water. The pellet was resuspended in a solution of 4% w/v sodium dodecyl sulfate (SDS) equal to 15 x the original sample wet weight and placed in a water bath at 100° C for 20 min. The suspension was then diluted with distilled water, to reduce its viscosity, and the pellet recovered by low speed centrifugation. The pellet was washed 5 × with distilled water to remove the SDS. After each wash any cell debris remaining in the supernatant were discarded. A dilute suspension of the washed pellet in distilled water was loaded onto a discontinuous sucrose gradient (50% w/v sucrose over 60% w/v sucrose) and centrifuged at 2,700 g in a swinging bucket rotor for 50 min at 4° C. Purified sheath was recovered as a pellet and washed with distilled water 4 x by low speed centrifugation before lyophilization. Visual observation by light microscopy revealed purified sheath fragments with no residual cellular material.

Scytonemin was extracted from colored sheath material into 90% acetone in water from an aqueous pellet. When acetone was removed under a stream of nitrogen, scytonemin was observed to crystallize and precipitate. Crystals were recovered by centrifugation or by settling overnight. Scytonemin was also extracted from the colored sheath by the method of Garcia-Pichel & Castenholz (1991) using methanol at 40° C followed by the addition of distilled water. Volumes were reduced with a rotary evaporator and crystals collected on GF/F glass fiber filters. Pigment crystals were removed from the filters by dissolving into tetrahydrofuran. The pigment extract was further purified on a Sephadex LH-20 column followed by high pressure liquid chromatography (HPLC) on a normal phase silica gel column (Beckman Instruments).

Analytical methods. Amino acid analysis was performed on a Pico-Tag amino acid analysis system as described by Bidlingmeyer *et al.* (1984). Sheath samples (2-100 µg) were hydrolyzed under vacuum in 6 M HCl at 110° C for 18 hours. Total protein was also determined with a modified ninhydrin assay (Rosen, 1957). Amino acids were released from the sheath polypeptide by hydrolysis in 6M HCl for 16 hours at 100° C. Monosaccharides were released from sheath samples by hydrolysis in 2 M trifluroacetic acid (TFA) in a sealed tube under nitrogen at autoclave temperature and pressure for 1, 2 or 3 hours. TFA was removed by reducing the sample to dryness under a stream of dry nitrogen. Initial examination of sheath carbohydrates was conducted by thin layer chromatography on silica gel G plates (solvent: ethyl acetate-pyridine-water; 12:5:3 v/v). The monosaccharides, both neutral sugars and uronic acids, were visualized by the naphthoresorcinol-sulfuric acid reagent No. 175 (Stahl, 1969). Neutral sugars were identified and quantified by gas liquid chromatography: mass spectrometry (GC-MS) of their alditol acetate derivatives (Blakeney *et al.*, 1983; York *et al.*, 1985) using a SP2330 capillary column (0.25 mm × 30 m). The injector and detector were held at 250° C, initial column temperature was 190° C increased at 10° C min^{-1} to 240° C and held there for 10 min. The alditol acetate derivatives were quantified as µg mg^{-1} of sample weight using the detector response value for each sugar. Mass spectrometric analysis was performed using a 5970 Mass Selective Detector (Hewlet-Packard) at 70 eV in the mass range m/z 40-550. Trimethyl silyl derivatives of neutral sugars and uronic acids were also prepared (York *et al.*, 1985). Glycosyl linkage analysis was performed by GC-MS of partially methylated alditol acetates (York *et al.*, 1985) using the same column as above, with an initial column temperature of 80° C increased at 30° C min^{-1} to 170° C then increased at 4° C min^{-1} to 240° C and held there for 10 min. Uronic acids were quantified using the method of Blumentkrantz and Asboe-Hansen (1973). Glucose was also measured enzymatically using ß-D-glucose oxidase as described by Sturgeon (1990). Glucose was released from the sheath by TFA hydrolysis for 1, 2 or 3 hours or by hydrolysis in 1 M HCl at 100° C for 16 hours in a sealed tube flushed with nitrogen. Analysis of sulfate content was conducted using the method of Terho & Hartiala (1970).

RESULTS

The procedure used for the isolation of the sheath yielded a purified sheath fraction showing no contamination by cell wall fragments when examined under the light microscope. Diaminopimelic acid, a marker for cell wall contamination, was not detected during amino acid analysis where the lower limit of sensitivity was approximately 0.01% of sample analyzed. Approximately 0.6% of the original wet weight of cultured material was recovered as dry weight of purified sheath. However, this is almost certainly a low value since the centrifugation steps in the purification sometimes resulted in the discard of sheath material.

The polypeptide content of pigmented and unpigmented sheath is expressed in terms of both mole % amino acids and total protein present as a percent of dry weight. The averaged amino acid analysis data from pigmented sheath and from unpigmented sheath (Table 1) show great similarity. Pigmented sheath contained 10.7% protein, by colorimetric assay, while unpigmented sheath contained 14.5% protein. Total percent protein as a summation of µg of each amino acid from amino acid analysis of unpigmented sheath

equaled 15.7%. The average total percent protein of both pigmented and unpigmented sheath is 12.9% of dry weight. The amino acid composition of *L. aestuarii* is unremarkable except that aspartic acid and alanine values are both high as compared to the sheaths of other cyanobacteria for which amino acid compositions are available. These two amino acids, aspartic acid (with a small polar side chain) and alanine (with a small non polar side chain), constitute 32.5% of the amino acid composition of the sheath.

Table 1. Amino acid composition of pigmented and unpigmented sheath, as mole %, from *Lyngbya aestuarii* Lieb. strain LM 8701. Sheath samples (2-100 µg) were hydrolyzed in 6 M HCl at 110° C for 18 hours under vacuum. Analyses were performed on a Pico-Tag amino acid analysis system (Bildingmeyer *et al.*, 1984). Total protein was also determined as % of dry weight using a modified ninhydrin assay. n is the number of separate sheath preparations which were averaged to yield the amino acid composition data presented.

	Pigmented (n = 2)	**Unpigmented** (n = 3)
Asp	16.5 mole %	15.9 mole %
Glu	8.4	6.8
Ser	9.0	9.4
Gly	7.6	9.1
His	0.3	0.4
Arg	2.2	3.1
Thr	9.5	9.7
Ala	16.6	16.3
Pro	2.7	2.5
Tyr	1.2	1.4
Val	5.7	5.6
Met	0.6	0.8
Ile	4.6	4.5
Leu	5.8	5.9
Phe	5.6	4.7
Lys	4.2	5.1

Carbohydrates in the sheath of *L. aestuarii* were initially identified using TLC methods. With the exception of galacturonic acid, all sugars detected by TLC were also identified when sheath samples were subsequently analyzed by GC-MS. Only one uronic acid, glucuronic, was identified during GC-MS analyses. However, on thin layer chromatograms two uronic acids, glucuronic and galacturonic, were identified by comparison against authentic standards. Why galacturonic was not detected during GC analysis has not been resolved. Recovery of uronic acids from polysaccharides can be variable after reduction for GC-MS (Quintero *et al.*, 1989).

Quantitative estimates for the various monosaccharides were obtained by GC (Table 2). Glucose in the sheath was also quantified with an enzymatic assay using ß-D-glucose oxidase which is specific for ß-D-glucose. Sheath samples were hydrolyzed in the same manner as was used for sample preparation for GC analysis (2 M TFA at autoclave temperature and pressure for 1 hour). The glucose concentration, when measured by using the enzymatic assay, was equivalent to 16.2% of the dry weight of pigmented

sheath and 20.7% of the dry weight of unpigmented sheath. An alternative hydrolysis in 1 M HCl for 16 hours at 100° C yielded glucose concentrations equivalent to 16.3% of the dry weight of pigmented sheath and 18.8% of the dry weight of unpigmented sheath. From these data, an average of 18.0% of the sheath dry weight is glucose. When the time of hydrolysis, using unpigmented sheath, in 2 M TFA at autoclave temperature and pressure, was extended to 2 and 3 hours, samples yielded glucose concentrations equivalent to 17.4% and 16.8% of sheath dry weight respectively.

Table 2. Quantification of monosaccharides by GC in pigmented and unpigmented sheath of *Lyngbya aestuarii* Lieb. strain LM 8701. Samples were hydrolyzed in 2 M TFA in a sealed tube under nitrogen at autoclave pressure for 2 hours. Monosaccharides were chromatographed on a SP2330 capillary column (0.25 mm × 15 m) as alditol acetate derivatives. The injector and detector were held at 250° C, initial column temperature was 190° C, increased at 10° C min^{-1} to 240° C, then held for 10 min. Alditol acetate derivatives were quantified as μg mg^{-1} of dry sample weight. Glucose and uronic acids were also measured using enzymatic and colorimetric assays respectively.

Glycosyl Composition	Pigmented	Unpigmented
Rhamnose	3.2 %	4.5 %
Fucose	0.81	0.67
Arabinose	2.7	1.8
Xylose	2.3	2.3
Mannose	4.3	6.1
Galactose	5.2	7.2
Glucose	8.8	15.0
Glucuronic acid	2.4	5.3

Glucuronic acid when quantified by GC represented 5.3% of the dry weight of unpigmented sheath. Total uronic acids, as a class, were also quantified in a colorimetric assay. When measured in glucuronic acid units unpigmented sheath contained 9.1% uronic acids and when measured in galacturonic acid units it contained 10.4% uronic acids (glucuronic and galacturonic acids produce slightly different amounts of chromogen in this assay). An average of 9.8% of the sheath of *L. aestuarii* is found to be uronic acids when quantified using this colorimetric assay.

The complexity of the polysaccharide sheath of *L. aestuarii* is evidenced by the number of identified linkages. Linkage analysis of the carbohydrates present in the sheath was performed by GC-MS analysis of partially methylated alditol acetate derivatives. Glucose, the most abundant sheath carbohydrate, which constitutes an average of 18% of the sheath dry weight when measured enzymatically, participates in 6 different linkage positions: Terminal-glucose, 3-glucose, 2-glucose, 4-glucose, 3,6-glucose, and 4,6-glucose. Galactose, the second most abundant sheath carbohydrate, representing an average of 6.2% of sheath dry weight was identified in 2 linkages: 3,4-galactose and 2,3-galactose. Mannose, which makes up an average of 5.2% of sheath dry weight was identified in 3 linkage positions: 6-mannose, 3,4-mannose and 4,6-mannose. Rhamnose, which on average makes up 3.9% of sheath dry weight, and arabinose, which on average constitutes 2.3% of sheath dry weight, were each identified in a single linkage position: 4-rhamnose and terminal-arabinose. No linkage positions were identified which involved xylose, which makes up 2.3% of sheath dry weight on average or fucose which makes up 0.7% of sheath

dry weight on average. However, these sugars must participate at least as terminal sugars. By adding one linkage position for each of these sugars, the total number of different linkages present is brought to fifteen.

DISCUSSION

The possibility that the sheath consists of more than one peptidoglycan or carbohydrate moiety cannot be resolved at this time. The complete insolubility of the sheath and inability to produce smaller soluble derivatives (Robbins, 1992) precludes any chromatographic or electrophoretic separation. Attempts to dissolve the sheath using 8M urea, phenol, formic acid, detergents or ion chelators did not produce any visible change in the sheath fragments or solubilization, therefore no chromatographic separation of sheath fractions was possible. However, the general quantitative reproducibility of individual analyses of sugars and amino acids from sheath of cultures of different age and different growth conditions (Robbins, 1992) suggests that the sheath may be a consistent, if highly resistant, polymer.

The amount of protein associated with untreated sheath varies widely, ranging from a low of 3.6% of dry weight in *Fischerella* sp. (Pritzer *et al.*, 1989) to a high of 22.6% in *Chlorogloeopsis* (Schrader *et al.*, 1982). It has not been possible to remove the protein associated with the purified sheath fraction by treatment with SDS or any other detergent, indicating a sheath peptidoglycan.

The carbohydrate and protein data on the composition of the sheath of *L. aestuarii* agree well with that reported by other authors for other cyanobacteria. In general, glucose is the most abundant monosaccharide of the sheath layer and is the most abundant sugar in the sheath of *L. aestuarii*. Several of the species of cyanobacteria in which the sheath layer has been investigated by other authors (Adhikary *et al.*, 1986; Weckesser *et al.*, 1987; Weckesser *et al.*, 1988; Pritzer *et al.*, 1989) were found to contain O-methyl sugars. However, no O-methyl sugars have been identified in the sheath carbohydrates of *L. aestuarii*. Further complicating sheath composition, an analysis for sulfate revealed a limited amount of sulfate present at 2.0% of total weight. This level of sulfate is similar to that found in *Synechocystis* (Panoff *et al.*, 1988). With the exception of the high alanine and aspartic acid content and the absence of O-methyl sugars the data for *L. aestuarii* are consistent with those from other cyanobacteria.

Analyses of the composition of sheath material are reported on a percent of dry weight basis. When the reported percent dry weight of all the known constituents of the sheath are totaled, they do not equal 100%. The average percent of dry weight which is of known composition is 55%. Thus, 45% of the sheath weight is unaccounted for. In contrast, when the composition of diffuse slime layers was examined the total percent of dry weight, which is of known composition, slightly exceeds 100% (Nakagawa *et al.*, 1987). It is unlikely that sheath material contains an undetected constituent which is responsible for this discrepancy. Instead, this anomaly is probably explained by the resistance of sheath material to hydrolysis under the conditions used during analysis. Weckesser *et al.* (1988) noted that the sheath of *Calothrix parietina* was not completely solubilized during the hydrolysis step prior to analysis.

More severe hydrolysis conditions with increased acid concentration, temperature or length of hydrolysis would be likely to release more monosaccharides from the

sheath polysaccharide. However, a greater loss of released monosaccharides also occurs. The added problem of the varying susceptibilities of these monosaccharides to degradation also exists (Torello *et al.*, 1980). An alternative hydrolysis protocol involving the use of liquid phase anhydrous hydrogen fluoride (Rorrer *et al.*, 1990), was not attempted in our investigation.

The information presented on the linkage structure of the carbohydrates from the sheath of *L. aestuarii* underscores the complexity of the sheath polysaccharide. No other data on linkage of carbohydrates from cyanobacteria sheaths or mucilage have been published to our knowledge.

Attempts to break the sheath carbohydrate into smaller oligosaccharides for detailed analyses were unsuccessful. A number of site specific enzymes (ß-glucuronidase, α-mannosidase) and general mixed enzyme systems (abalone acetone powder, snail acetone powder and Driselase (a mixture of enzymes from the fungus *Irpex lacteus* which contains both endo and exo-hydrolases) failed to produce smaller oligosaccharides under digestive conditions.

The function that proteins play in sheath structure is not known. However, proteins appear to be an intrinsic part of sheath structure. Even after drastic treatments to remove associated proteins (boiling in SDS for 20 minutes) the sheath fraction retained a protein component. This implies the presence of one or more glycoproteins in the sheath. The sheath of *L. aestuarii* contained 12.9% intrinsic protein or glycoprotein and is within the range of values reported in the literature for other cyanobacteria.

Changes in temperature, light intensity and the availability of combined nitrogen have been shown to affect sheath composition or morphology (Findlay *et al.*, 1970; Evans & Foulds, 1976; Tease & Walker, 1989). When *L. aestuarii* was grown under two light regimes to produce pigmented and unpigmented sheath samples, no obvious changes in sheath morphology were observed. Data from these two culture conditions have been reported separately, then averaged for use in comparisons with data generated by other authors. This approach, which may more accurately reflect sheath composition under field conditions, where the top millimeters are pigmented and shade the underlying non pigmented layers, is not without difficulties. Some parameters of sheath composition were more affected by the high-light regime than others. Amino acid composition was least affected, while total percent protein and total percent carbohydrate were lower in pigmented than in unpigmented sheath samples (Tables 1 and 2).

Scytonemin appears to play no role in sheath integrity. Its ease of removal indicates that it is not covalently bound to the carbohydrate. The unchanged sheath composition and integrity, with and without scytonemin, suggest that it plays no role in sheath structural chemistry. The sheath appears to play the role of a support matrix, allowing the pigment to be retained on the filament surface and thus to serve as a UV shield (Garcia-Pichel & Castenholz, 1991). Structural analysis of purified pigment by NMR identified two components, a monosubstituted phenol fragment and a disubstituted phenyl ring fragment (Alvi & Robbins, unpublished data). These data are in agreement with the full structure of scytonemin published by Proteau *et al.* (1993). This suggests that the pigment of *Lyngbya* is indeed scytonemin or a very similar pigment.

A number of questions about sheath architecture still remain. Among them are: the nature and function of glycoproteins within the sheath, the sequence of monosaccharides in the backbone and side of chains of sheath polysaccharide(s), the potential cross-linking of polymers to one another and what aspect of sheath structure confers resistance to degradation. These questions address sheath structure and function at a level above compositional analysis. However, the chemical resistance of the sheath precludes a

quick and simple solution. The groundwork on composition does point out the high degree of complexity found in the sheath of *L. aestuarii* and in cyanobacterial sheaths in general.

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ANNONCE DE CONGRÈS

17e COLLOQUE de l'Association des Diatomistes de Langue Française (ADLaF)
Luxembourg, 8-11 septembre 1998

L'accueil se fera le mardi 8 septembre à 9h00 du matin au Bâtiment des Sciences du Centre Universitaire de Luxembourg. Les chercheurs sont invités à présenter leurs résultats sur les diatomées sous forme de communications, de posters ou de vidéos. Les thèmes abordés sont libres et variés : diatomées fossiles, actuelles, d'eau douce, marines, saumâtres, systématique, écologie, paléoécologie, qualité des eaux, mouvement des diatomées, etc. L'Assemblée générale de l'Association se tiendra le jeudi 9 septembre en fin d'après-midi. La journée du vendredi 11 sera consacrée à l'observation microscopique d'échantillons et à l'identification du matériel apporté par les participants au Colloque.

Une visite de la ville de Luxembourg le 8 au soir ainsi qu'une excursion au Château médiéval de Vianden (« la perle des Ardennes ») et dans la région du Müllerthal (« Petite Suisse ») l'après-midi du 9 septembre permettront d'apprendre à mieux connaître le Grand-Duché de Luxembourg. L'hébergement se fera dans les hôtels de la ville de Luxembourg ou en auberge de jeunesse.

Inscription souhaitée avant le 1er juin 1998
Frais d'inscription : 1500 Flux (environ 250 FF)
Date limite de réception des résumés des communications et posters : 1er juillet 1998

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Cryptogamie, Algologie, *Cryptogamie, Bryologie-Lichénologie* et *Cryptogamie, Mycologie* publient des articles originaux ou des articles de synthèse en systématique, biologie et écologie des cryptogames (algues, bryophytes et lichens, champignons, respectivement). Les manuscrits rédigés en français, ■ anglais, en allemand, en espagnol et ■ italien, sont pris en considération dans la limite des possibilités de la Rédaction à trouver des lecteurs compétents dans ces langues. Les auteurs n'écrivant pas dans leur langue doivent, avant soumission, faire relire leur texte par une personne dont la langue du manuscrit est la langue maternelle. Il n'y a pas de limite au nombre de pages. La publication d'un article sera facilitée si les auteurs suivent soigneusement les instructions ci-après.

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AJISAKA T., NORO T., TRONO Jr. G.C., YOUNG-MENG CHIANG & YOSHIDA T., 1994 — Several *Sargassum* species (subgenus *Sargassum*) in East Asia with furcately branching leaves. *In*: Abbott I.A. (ed.), *Taxonomy of Economic Seaweeds*. IV. La Jolla, California Sea Grant College, University of California, pp. 9-22.

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